"SYNTHESIS OF CERTAIN SCHIFF BASES OF 7-HYDROXY-3-FORMYL CHROMONE AS THYMIDINE PHOSPHORYLASE INHIBITORS AND EVALUATION OF OTHER POSSIBLE BIOLOGICAL ACTIVITIES"

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OCTOBER 2016

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INTRODUCTION

Carcinoma is the leading cause of premature death in the world. Due to the rapid advancement in cancer diagnosis and therapy, the life expectancy and survival rate of cancer patients have shown improvement. However, the overall clinical outcome of cancer is still unsatisfactory due to its high invasive and metastatic properties. In order to achieve therapeutic success in cancer patients, there is still room to develop new treatment strategies that target at various stages of tumour progression. A flurry of scientific investigations has identified angiogenesis as one of the important processes in cancer development. Angiogenesis was found to promote tumour growth and metastasis. Therefore antiangiogenesis might be an effective cancer treatment strategy.

Among several angiogenic activators, thymidine phosphorylase has been recognized as an important angiogenic protein that is frequently overexpressed within solid tumours. In recent years, emerging data provided convincing evidence that TP and its metabolic product, 2-deoxy-D-ribose (2DDR) stimulate the secretion and/or expression of many angiogenic factors, such as MMPs and VEGF, which trigger a signalling cascade to induce endothelial cell migration, proliferation, elongation and sprouting. These events eventually lead to angiogenesis and cancer metastasis. Therefore, TP has been implicated as a potential target for the development of chemotherapeutic agents.[1]

Thymidine phosphorylase[2-11]

Thymidine phosphorylase (TP) was first discovered in 1954 as a key enzyme of the pyrimidine salvage pathway which catalyzes the conversion of thymidine and 2- α -deoxyuridine to their respective bases (thymine and uracil) and 2-α-D-deoxyribose-1-phosphate (2DDR-1P).

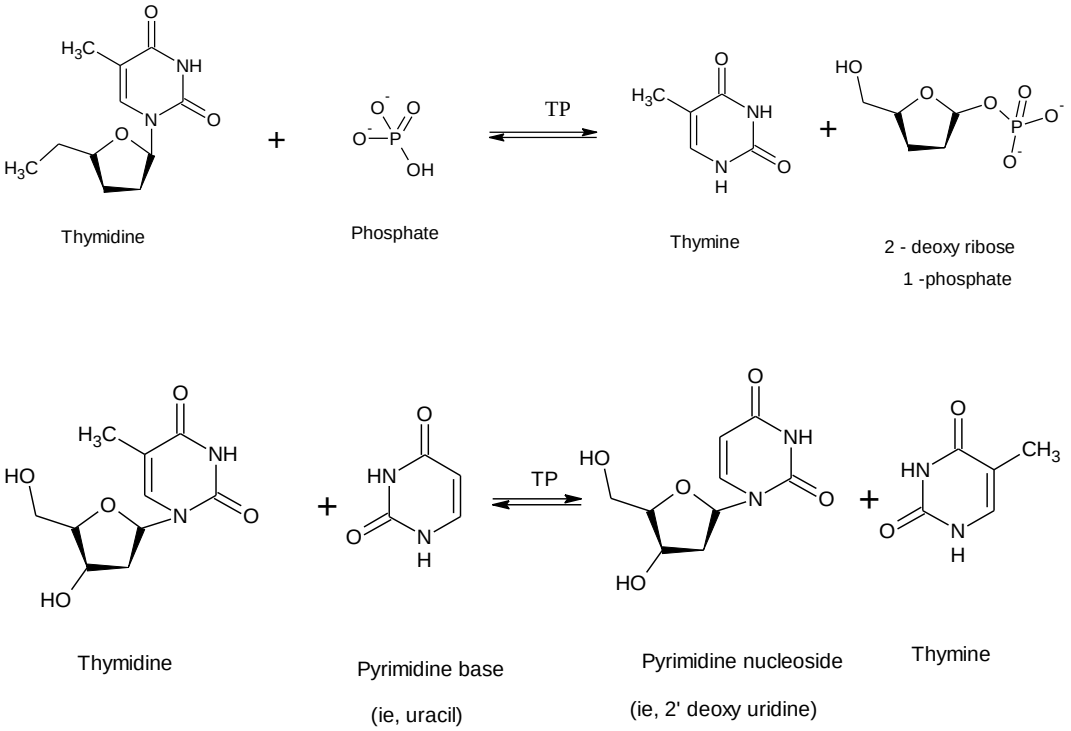


Figure 1 :*Enzymatic reaction catalyzed by TP.*TP catalyzes the reversible conversion of thymidine to thymine and 2DDR-1P.TP also has deoxyribosyltransferase activity by which the deoxyribosyl moiety is transferred from one pyrimidine base to another, resulting in the formation of a new nucleoside.

This reaction is reversible; however, the most important metabolic function of TP is catabolic. TP also has deoxyribosyl transferase activity by which the deoxyribosyl moiety is transferred from a pyrimidine nucleoside to another pyrimidine base, resulting in the formation of a new pyrimidine nucleoside. Besides natural 2 α-deoxynucleosides, TP also recognizes several pyrimidines or pyrimidine nucleosides with antiviral and antitumoral activity, such as 5-(E)-(2-bromovinyl)-2α-deoxyuridine (BVDU), 5-trifluorothymidine (TFT), 5-fluorouracil (5FU), and 5-fluoro-5 α-deoxyuridine (5αDFUR), an intermediate metabolite of Capecitabine, which is clinically used against metastatic breast and colon cancer.

In 1987, a so-called ‘‘new’’ protein was isolated from human blood platelets. This protein was believed to stimulate endothelial cell growth because it increased the [^{3}H]-thymidine uptake and was therefore named ‘‘platelet-derived endothelial cell growth factor (PD-ECGF).’’ PD-ECGF was also shown to induce endothelial cell migration *in vitro* and angiogenesis *in vivo.* A few years later, it was reported that recombinant PD-ECGF has TP activity. Moreover, analysis of the amino acid sequence of both proteins revealed that PD-ECGF and TP are identical. This leads to the conclusion that the observed increased thymidine uptake was an artifact, caused by the TP activity of PD-ECGF.

A third role for TP has also been described and in this context TP is called gliostatin. In 1992, gliostatin was extracted from human neurofibroma. This protein inhibits the growth of both astrocytes and glial tumor cells. Thus, TP, PD-ECGF and gliostatin are all synonyms that refer to the same, identical protein.

STRUCTURE OF TP

In the mid-1970s TP was purified from both *Escherichia coli* and *Salmonella typhimurium*. Several years later, human TP was extracted from the amniochorion. The amino acid sequence of TP is highly conserved during evolution. For example, human TP shares 39% sequence identity with *E. coli* TP.

TP functions as a homodimer consisting of two identical subunits (Fig. 2), with a dimer molecular mass ranging from 90 kDa in E. coli to 110 kDa in mammals. Detailed structural information on TP was first provided in 1990 by Walter et al. who solved the crystal structure of *E. coli* TP. This analysis revealed that each subunit contains a large mixed α-helical and β-sheet domain (α/β domain) separated from a smaller α-helical domain (α-domain) by a large cleft. The active site consists of the thymine-binding site in the α-domain and the phosphate-binding site across the cleft in the

α/β domain. The finding that both sites were about 8Å apart immediately suggested that a hinge motion of one domain relative to the other was necessary to generate a closed conformation of the enzyme.

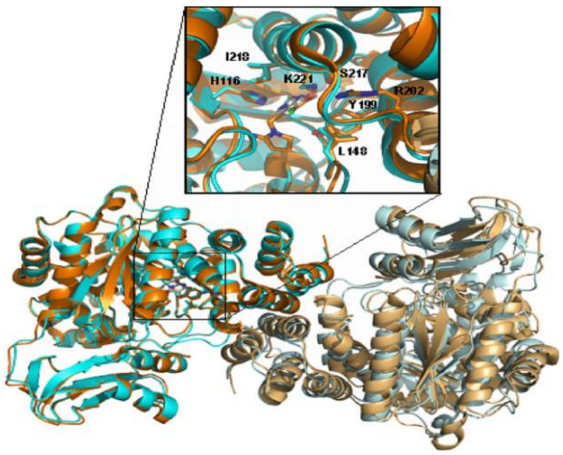


Figure 2. Ribbon representation of human TP showing the dimeric structure and a detail of the active site (boxed) containing either the TP inhibitor (C atoms in orange) or the thymine product (C atoms in white).

It took several more years to have the structure of human TP solved because many crystallization trials failed to produce well-diffracting crystals. Thus, Spraggon et al. reported crystals of human TP for which, despite using a synchroton X-ray source, diffraction was limited to 3.5Å resolution. Finally, in 2004, Norman et al. successfully solved at 2.1Å resolution the structure of human TP in complex with the small and potent inhibitor 5-chloro- 6-[1-(2-iminopyrrolidinyl)methyl] uracil (TPI).

In 2006, El Omari et al. managed to determine the structure of unproteolyzed human TP at 2.3 Å resolution with the aid of the small-molecule inhibitor KIN59, which helped to obtain good quality diffracting crystals. In Figure 2, a ribbon representation of human TP is shown, clarifying the dimeric structure and a detail of the active site containing either the inhibitor TPI or the thymine product. As kinetic studies of *E.coli* TP have shown that phosphate is the first substrate that binds to TP

whereas 2DDR-1P dissociates last from the enzyme, this finding suggests that after product release, thymine is able to reassociate with the unliganded enzyme and stabilize the closed conformation, which may explain the mechanism of noncompetitive product inhibition.

The Physiological Role of TP

TP is found in many normal tissues and cells, with high levels in macrophages, stromal cells, glial cells, reticulocytes, some epithelial tissues of the digestive tract (oesophagus and the rectum), salivary gland, brain, bladder, spleen, lymph, and the lungs. Within the cell, TP is present in both the cytoplasm and the nucleus. Blood platelets are one of the richest sources of TP, which suggests a role for the enzyme in wound healing. TP activity is also detected in plasma and serum, where its presence is probably due to blood platelet damage or cell turnover. Furthermore, TP plays an important role in the female reproductive cycle. Large quantities of TP are found in the placenta, where two alternative forms of the protein are detected.

TP Overexpression in Cancer

Thymidine phosphorylase is a highly expressed protein in many solid human tumours, and the level of expression is associated with tumour neovascularization, invasiveness and metastasis. TP is predominantly expressed in hypoxic regions of solid tumours, promoting tumour growth by angiogenesis, metastasis and suppressing apoptosis. TP is also known as platelet-derived endothelial cell growth factor (PD-ECGF), a novel angiogenic protein, distinct from other angiogenic growth factors, since it exerts its actions through its enzymatic activity and inhibitors prevent the angiogenic activity of TP. The dephosphorylated product, D- 2-deoxyribose has been shown to have chemotactic activity *in vitro* and angiogenic activity *in vivo* and is considered to play a key role in

the invasiveness and metastasis of TP expressing solid tumours . Since TP is over-expressed in tumours, the protein is an attractive cancer chemotherapy target for selective inhibition of TP-dependent angiogenesis and subsequent inhibition of tumour growth. In addition, specific inhibitors of human TP could also enhance the efficacy of thymidine analogues such as 5-fluoro-2α-deoxyuridine and 5-iodo-2α-deoxyuridine, which would no longer be metabolized and inactivated by TP. The classical TP inhibitors include 6-amino-5-bromouracil(6A5BU) and 7-deazaxanthine (7-DX) which was the first purine derivative to display inhibition of both E.*coli* TP and angiogenesis in a chorioallantoic membrane assay .Indeed, a potent inhibitor of TP, 5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]uracil (TPI) entered clinical trials in combination with 5-trifluoromethyl- 2α-deoxyuridine as an orally available anticancer treatment under the name of TAS-102.

Increased TP expression in tumor tissues compared to corresponding nonneoplastic tissue was found in breast, bladder, gastric, colorectal, lung, esophageal, and cervical cancers but not in cancers of the liver,common bile duct, and the thyroid. TP expression is not only upregulated in solid tumors, elevated levels of TP were also observed in lymph nodes of patients with classical Hodgkin lymphoma where TP levels increased with disease progression. These data identify TP as a potential target for the immunotherapy of hematological tumors.

Numerous studies on cancer patients have examined the relation between TP expression and microvessel density, tumor grade, stage, metastasis and prognosis. In these studies TP was measured by RT-PCR, immunohistochemistry, or by activity assays. Generally, TP expression is correlated with higher microvessel density, higher tumor stage and more metastasis. An association of TP with tumor grade is evident in bladder,

cervical, and renal cell cancer, but not in the other investigated cancers. Furthermore, in most cases, TP appeared to be associated with poor prognosis, although there are conflicting reports for some cancers. For example, seven of the nine studies on colon cancer reported a significant correlation between TP and bad prognosis, while Saito et al. demonstrated that TP is associated with good prognosis. These discrepancies might be caused by differences in the histological type of cancer, stage (early versus advanced stage of disease), number of patients examined, assay for TP and different methodology for the evaluation of the immunohistochemistry results.

Tumors are heterogeneous tissues consisting of unknown variable contributions of tumor, stromal, and infiltrating cells. Besides tumor cells, also endothelial cells, fibroblasts, lymphocytes and especially tumor-associated macrophages (TAM) express TP. TAM are thought to play a key role in stimulating tumor growth and metastasis through the production of various growth factors, proteinases, chemokines, and cytokines. High levels of TP have been demonstrated in TAM of melanoma, gastric, glioblastoma, breast, colon, astrocytic,uterine, endometrial and prostate cancer. In gastric adenocarcinoma, astrocytic tumors, breast, and uterine endometrial cancer, TP expressed in macrophages has been suggested to be correlated with microvessel density and to play an important role in tumor invasiveness.

Elevated TP levels are not only found in the tumor tissue but also in the plasma of cancer patients. Already in 1977, Pauly et al. demonstrated that cancer patients had much higher TP activity in the plasma than healthy individuals. More recent data indicate that plasma TP concentrations in cancer patients may have a prognostic value. In uterine cervical cancer high serum TP levels correlate with clinical stage, tumor

size, lymph node metastasis and an extremely poor prognosis. High TP concentrations in the blood are also associated with depth of tumor invasion and poor response to treatment in patients with esophageal squamous cell carcinoma. Furthermore, in patients with colorectal cancers, the TP serum level is suggested to be a novel marker to predict occurrence of hematogenous metastasis.

The inhibition of TP may result in reduction of tumor growth and metastasis and it also potentiates the antiproliferative effect of nucleoside drugs such as 5-(E)-(bromovinyl)-2α-deoxyuridine, 2α-deoxy-5-trifluoromethyluridine, 2α-deoxy-5-iodouridine and 5-fluoro-2α-deoxyuridine which are substrates of TP. As follows from the survey of literature, the inhibition of TP represents a promising target in cancer chemotherapy. Schiff bases of 3 formylchromone has been already reported as thymidine phosphorylase inhibitors.

TP AND TUMOR DEVELOPMENT[12-16]

A. Role of TP in Angiogenesis

Angiogenesis is the formation of new blood vessels from preexisting vessels and it is essential for organ growth and repair. However, it is well known that this is a vital step in the process of cancer growth. Thus, angiogenesis inhibitors are believed to be potential candidates for blocking cancer growth.

In particular, thymidine phosphorylase (TP) is a pro-angiogenic factor which catalyzes the reversible phosphorolysis of thymidine into thymine and 2α-deoxy-D-ribose 1-phosphate.The 2α-deoxy-D-ribose 1-phosphate undergoes further dephosphorylation to produce 2-deoxy-D-ribose which stimulates the secretion of vascular endothelial growth factor (VEGF). VEGF activates a number of processes including endothelial cells

for secretion of matrix metalloproteinases, proliferation, and migration of endothelial cells to tumor tissue. These actions result in fast generation of new blood vessels and cancer metastasis.

1. **TP stimulates endothelial cell migration**

The molecular mechanisms through which TP and 2DDR stimulate endothelial cell migration *in vitro* are not completely understood. Hotchkiss et al revealed that TP and 2DDR affect endothelial cell migration through activation of integrins and their downstream signaling pathways. In human umbilical vein endothelial cells (HUVEC), it was shown that both TP and 2DDR stimulate the formation of focal adhesions and the phosphorylation of tyrosine 397 of focal adhesion kinase (FAK). FAK is a nonreceptor protein–tyrosine kinase that is recruited to focal adhesions by integrin. Thus, FAK plays an important role in endothelial cell attachment and migration.Hotchkiss et al. also demonstrated that VEGF, TP, and 2DDR all stimulate HUVEC migration, although through different integrins.

2. **TP induces the expression and/or secretion of other angiogenic factors**

Various studies have demonstrated that TP and 2DDR promote the expression and secretion of several angiogenic factors. Human bladder carcinoma cells transfected with TP (RT112-TP) secrete higher amounts of VEGF, interleukin-8, and MMP-1 than mock transfected RT112 cells in the presence of thymidine. RT112-TP cells incubated with thymidine also showed an elevated expression of heme oxygenase-1 (HO-1), HO-1 is an enzyme that catalyzes the degradation of heme to carbon monoxide, iron, and biliverdin. The expression of HO-1 can be induced by hypoxia, cytokines, and several angiogenic factors such as VEGF and stromal cell derived factor-1 (SDF-1). Recent data indicate that HO-1 also possesses proangiogenic properties: it promotes endothelial cell proliferation, protects

endothelial cells from apoptosis, and induces the secretion of several angiogenic mediators. Brown et al. suggested that 2DDR is a strongly reducing sugar that may generate oxygen radical species during the early stages of protein glycation. It was hypothesized that 2DDR binds to an amino group (preferentially at a lysine, arginine or the N-terminal amino acid) of a protein during a nonenzymatic reaction, the so-called Maillard reaction. This may lead to the formation of a Schiff base, which can then rearrange to an α-hydroxyketone. This unstable reaction intermediate autoxidizes during which reactive free oxygen radicals are produced. Thus, through the formation of 2DDR, TP may induce oxidative stress in TP-overexpressing tumor cells causing these cells to secrete angiogenic factors, such as VEGF.

B. TP Induces Metastasis

TP was found to increase the metastatic potential of several experimental and human tumors. Moreover, in various cancers high TP expression correlates with metastasis. Takao et al. demonstrated that TP-expressing KB carcinoma cells show more basement membrane invasion than their mock-transfected counterparts. The stimulation of metastasis by TP-overexpressing cells could be dramatically inhibited by the TP inhibitor TPI or by 2-deoxy-L ribose (2DLR), a stereoisomer of 2DDR.

C. TP Protects Cancer Cells Against Apoptosis

A correlation between TP expression and apoptosis was first demonstrated *in vitro* by using human epidermoid carcinoma KB cells. KB cells transfected with TP (KB/TP) were resistant to hypoxia-induced apoptosis. This advantage was abrogated when the cells were treated with TPI, which inhibits the enzymatic activity of TP, leading to the conclusion that the enzymatic activity of TP is indispensable for protection against

hypoxia-induced apoptosis. Also the metabolites of the TP reaction, 2DDR and thymine, partially prevented hypoxia-induced apoptosis in KB cells.

TP INHIBITORS[17-22]

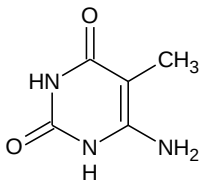
In 1971, Judah Folkman postulated that tumor growth is angiogenesis-dependent and that tumor development and metastasis could be abolished by blocking the tumor blood supply. Currently, anti-angiogenic drugs, such as the VEGF-antibody Bevacizumab (Avastin) and the kinase inhibitors Sorafenib (Nexavar), and Sunitinib (Sutent) are being used in cancer treatment while dozens of other anti-angiogenic molecules are evaluated clinically. However, the benefits from these anti-angiogenic therapies are at the best temporary and mostly followed by resistance development of the tumor. Although tumor resistance may be caused by various mechanisms such as poor pharmacokinetics, limited drug uptake, increased drug efflux, and mutation of the target proteins, tumor resistance may also be caused by circumvention of the angiogenic blockade by activation and/ or upregulation of alternative pro-angiogenic pathways in the tumor. For example, a study on glioblastoma patients treated with the VEGF receptor inhibitor cedinarib (Recentin) showed that the tumors evaded the anti-angiogenic therapy by upregulating the angiogenic fibroblast growth factor-2 (FGF-2) and stromal cell derived factor-1α (SDF-1α). Therefore, there is an urgent need to develop anti-angiogenic drugs directed at different angiogenic targets.

As TP plays a fundamental role in cancer angiogenesis, many laboratories have tried to synthesize potent TP inhibitory drugs. Some of these molecules have been tested preclinically and clinically, but currently no product has been approved yet for clinical use.

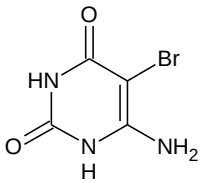
A. Pyrimidine Analogues

For more than 30 years the only compounds known to inhibit TP were uracil derivatives, such as 6-aminothymine (6AT) and 6-amino-5-bromouracil (6A5BU). These molecules have 50% inhibitory concentration (IC_{50}) values against the enzyme in the submicromolar range. When it became clear that TP is not only an enzyme involved in the nucleoside salvage pathway but is also implicated in angiogenesis, numerous laboratories aimed at synthesizing more potent TP inhibitors.

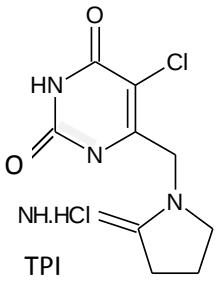
In 2000, Fukushima et al. identified ‘‘5-chloro-6-[1-(2 iminopyrrolidinyl) methyl] uracil hydrochloride (TPI)’’ (Fig. 3), the most potent inhibitor of human TP so far, with an IC_{50} value of 35 nM. This molecule was shown to abrogate several biological actions of TP. For example, TPI inhibited TP-induced angiogenesis in the mouse dorsal air sac assay. It also significantly reduced the tumor growth rate and microvessel density and increased the apoptotic index of KB/TPxenografted tumors. Furthermore, oral administration of TPI suppressed macroscopic liver metastasis of highly metastatic KB/TP cells and also the level of human β -globin as a molecular marker of micrometastasis in the livers of the mice. The fact that TPI is orally bio-available and has a strong nanomolar inhibitory activity against TP suggests that this molecule might be a promising antitumor agent.



6-Amino Thymine



6- Amino 5-Bromo Uracil



TPI

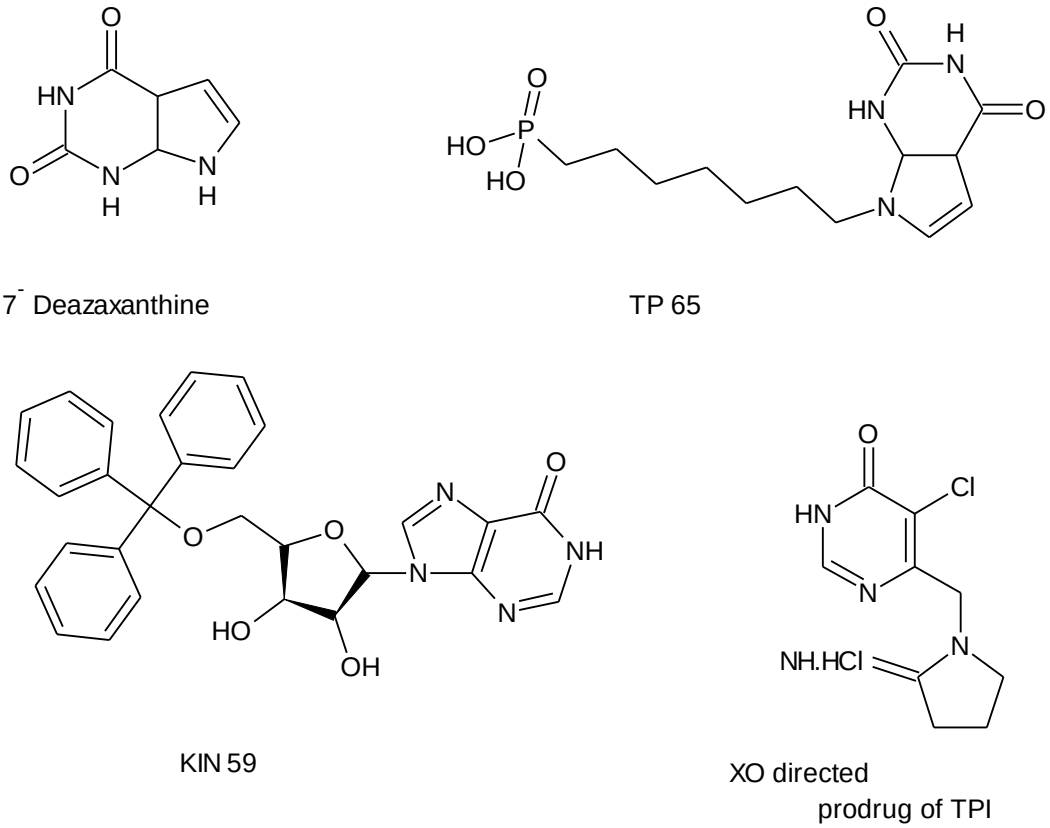


Figure 3. Chemical structure of some illustrative inhibitors of TP.

B. Purine Analogues

Balzarini et al described 7-deazaxanthine (7-DX) (Fig. 3) as the first purine derivative with inhibitory activity against a pyrimidine nucleoside phosphorylase (i.e. TP). The three-dimensional structure of *E. coli* TP was used for the rational modelling and design of 7-DX, which can be regarded as a pyrimidine at which a second ring was added to create extra stabilization. 7-DX not only efficiently inhibited the enzymatic activity of TP; it was also able to prevent neovascularization in the CAM assay. The available crystal structure of *E. coli* TP has also led to the rational design of compounds that interact both with the thymine and the phosphate-binding site, the so-called multisubstrate analogue inhibitors of TP. These types of molecules consist of a base, interacting with the nucleoside-

binding site and a phosphonate moiety that may bind to the phosphate-binding site. The distance between the thymine and the phosphate-binding site of *E. coli* TP is estimated to be around 10A˚, therefore the thymine and the phosphonate moiety of these novel inhibitors were linked to each other with a spacer of 6–9 methylene entities. These compounds interact with both substrate-binding sites, and thus ‘‘freeze’’ the enzyme in an open, inactive conformation. TP65, which contains an alkyl phosphonate moiety covalently linked to 7-DX (Fig. 3), is such a multisubstrate inhibitor of TP, with an IC_{50} value in the micromolar range. This molecule could also abrogate biological activities of TP, such as angiogenesis in the CAM assay and the formation of microvascular sprouts from endothelial cell aggregates in a fibrin gel.

Another purine derivative that inhibits TP is 5α-O-tritylinosine (KIN59). KIN59 consists of a purine base (hypoxanthine), a ribose sugar and a trityl group at the 5α-position of the ribose. The trityl group of KIN59 has proven to be crucial for its inhibitory activity against TP and its anti-angiogenic effect in the CAM assay. KIN59 is in several ways a very unusual TP inhibitor. In the CAM assay, KIN59 not only prevented the formation of new blood vessels but also promoted the degradation of small pre-existing immature blood vessels. This effect was not due to unspecific cell toxicity. Furthermore, in contrast to all previously described TP inhibitors, this molecule does not compete with the natural substrates for binding to either the nucleoside or the phosphate-binding site of TP, but interacts with a new, yet unknown, allosteric site of the enzyme in a non-competitive fashion.

C. Prodrugs of TP Inhibitors

Reigan et al. have explored a xanthine oxidase (XO) prodrug strategy. XO activity and expression are increased in hypoxic conditions. Moreover, increased XO activities are found in colorectal and prostate

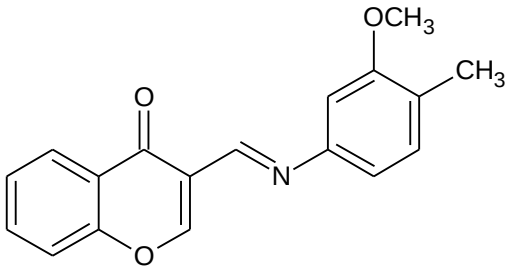
tumors as compared to their corresponding normal tissues. Therefore, 2'-nitro prodrugs of potent 2'-aminoimidazolyl TP inhibitors were developed. These prodrugs may become selectively activated by XO in the tumors and thus may exert their TP inhibitory activity specifically within the hypoxic regions of the tumors. Also XO sensitive prodrugs of 6A5BU, 7-DX and TPI have been synthesized. The *in vivo* efficacy of these prodrug molecules remains to be investigated.

LITERATURE REVIEW

SCHIFF BASES OF 3 FORMYL CHROMONE

As Thymidine phosphorylase inhibitors

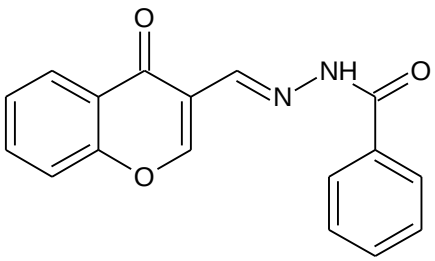
K.M Khan., et al. synthesized a series of Schiff bases of 3 formyl chromone and evaluated their thymidine phosphorylase inhibitory activity.[3]



3-{[(3-Methoxy-4-methylphenyl) imino] methyl}-4Hchromen- 4-one exhibited the highest inhibitory activity.

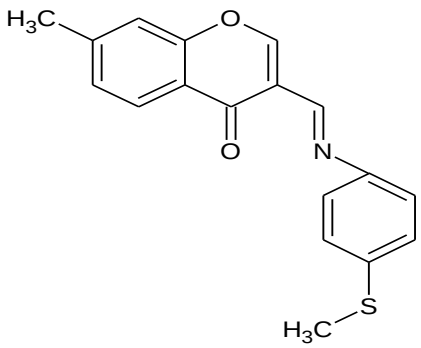
As anti cancer agents

Shadia et al., synthesized Schiff base derived from 3-formylchromone and benzohydrazide and its Ni(II), Zn(II), Ru(II), Ru(III), Pd(II), Pt(II) and Ag(I) complexes have been tested as anticancer agents against the human breast cancer (MDAMB 231) and human ovarian cancer (OVCAR-8) cell lines.[23]



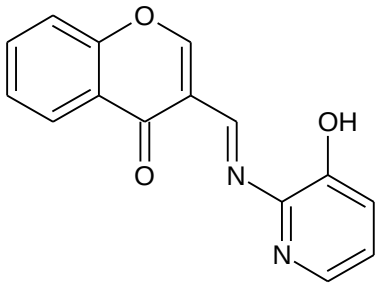
The Ag(I) complex showed promising efficacy.

Osowole et al., synthesized Mn(II), Co(II), Ni(II), Cu(II) and Pd(II) complexes of the Schiff bases obtained by condensation of 3-formyl-6-methylchromone and 4- methyl thioaniline and evaluated its cytotoxic activity against both MCF-7(human breast adenocarcinoma) and HT-29 (colon carcinoma) cells.[24]



The cytotoxic study shows that the Cu(II) complex has the best *in-vitro* anticancer activity against both MCF-7 (human breast adenocarcinoma) and HT-29 (colon carcinoma) cells, with IC_{50} values of 9.78 µM and 17.02 µM respectively.

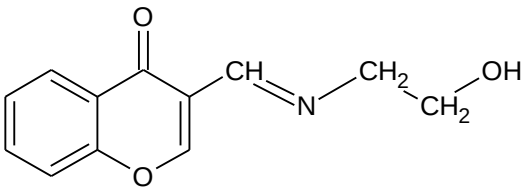
Kavitha et al., synthesized Pd(II) complexes of 3-formyl chromone Schiff bases and the cytotoxicity of complexes was tested on three cell lines(murine macrophage cell line Raw 264.7, human breast cancer cell line MCF-7 and human colon carcinoma cell line (COLO 205).[25]



Among all complexes pyridine ring containing complexes are potent.

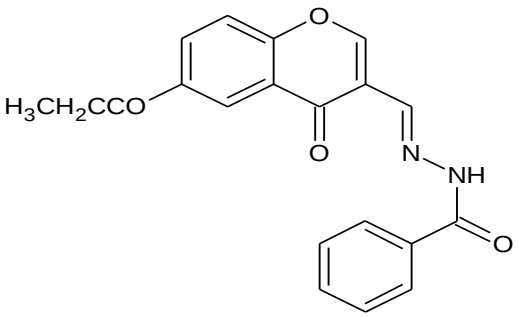
DNA binding studies

Farukh et al., synthesized new chromone Schiff base organotin(IV) complexes {dimethyltin dichloride, diphenyltin dichloride and triphenyltin chloride}. The in vitro DNA binding profile of complexes was carried out by absorption, fluorescence spectroscopy and viscosity measurements. [26]



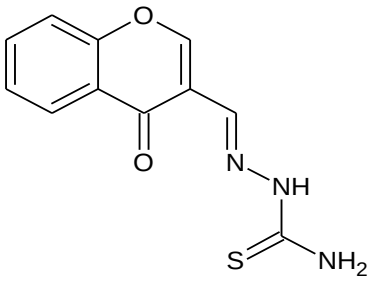
The corroborative results of DNA binding studies revealed that the complexes exhibit electrostatic mode of binding preferably via oxygen of sugar-phosphate backbone of DNA helix.

Ju Wang et al., synthesized novel 6-ethoxy chromone-3-carbaldehyde benzoyl hydrazone and its Ln(III) complexes, [Ln= Sm (1), Eu (2), Gd (3), Tb (4)]. The DNA-binding properties of the Eu(III) and Sm (III) complexes were investigated using UV Vis absorption spectroscopy, fluorescence spectroscopy and viscosity measurement. [27]



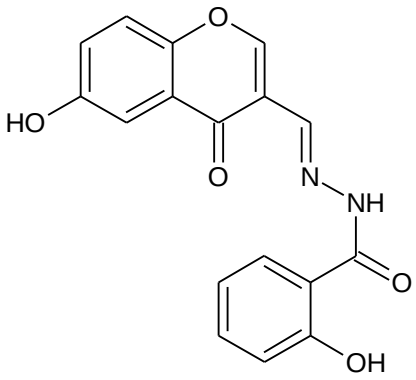
All the experimental evidences indicate that the two complexes can bind to CT-DNA via an intercalation mechanism.

Yong Li et al., reported the synthesis of 3-carbaldehyde chromone thiosemicarbazone and its Copper (II), Zinc (II) and Nickel (II) complexes . Interactions of ligand and Cu(II), Zn(II) and Ni(II) complexes with DNA were investigated by spectral and viscosity studies.[28]



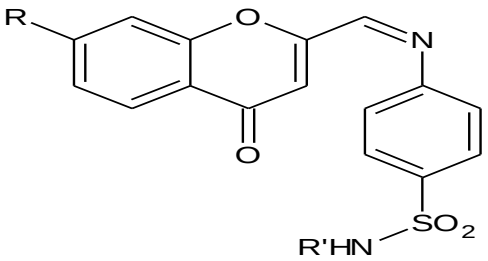
DNA binding studies indicate that the investigated compounds bind to DNA via an intercalation binding mode and Zn(II) complex binds to DNA most strongly.

Wang BD et al., reported the synthesis of a new ligand, 6-hydroxy chromone-3-carbaldehyde-(2′-hydroxy) benzoyl hydrazone by condensation of 6-hydroxy-3-carbaldehyde chromone (CDC) with 2-hydroxy benzoyl hydrazine along with its four rare earth Ln(III) complexes [Ln = La(1), Sm(2), Dy(3), Eu(4)]. Spectrometric titration, ethidium bromide displacement experiments, and viscosity measurements indicate that Eu(III) complex and ligand, especially the Eu(III) complex, strongly bind with calf-thymus DNA, presumably via an intercalation mechanism.[29]



As carbonic anhydrase inhibitors.

Luca Puccetti et al., synthesized a series of Schiff bases by reaction of 3- formyl-chromone or 6-methyl-3-formyl-chromone with aromatic sulfonamides such as sulfanilamide, homosulfanilamide, 4-aminoethyl-benzene sulfonamide, a pyrimidinyl-substituted sulfanilamide derivative, sulfaguanidine and 4-amino-6-trifluoromethyl-benzene-1,3-disulfonamide. The zinc complexes of these sulfonamides have also been obtained. The new derivatives and their Zn(II) complexes were investigated for the inhibition of four physiologically relevant isozymes of carbonic anhydrase : the cytosolic isoforms I and II, as well as the tumor-associated, transmembrane isozymes CA IX and XII.[30]

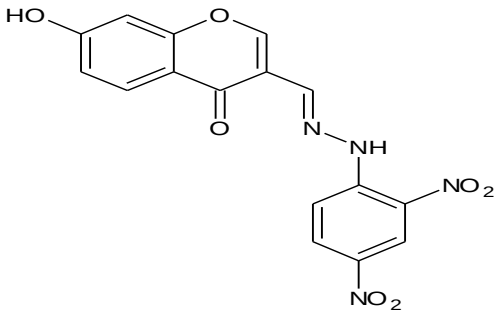


R= H, Me
R' = H, C(=NH)NH 2

Except for the sulfaguanidine-derived compounds which were devoid of activity against all isozymes, the other sulfonamides and their metal complexes showed interesting inhibitory activity.

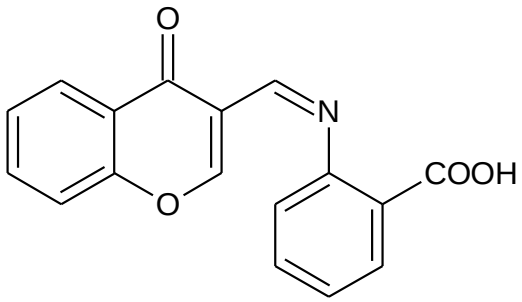
As antimicrobial agents

Patil et al., synthesized Schiff bases of 7-hydroxy-3-formyl chromen-4-one. All the synthesized compounds were evaluated for antimicrobial activity against both gram positive and gram negative organisms. [31]



The Schiff base synthesized from 2,4-dinitro phenyl hydrazine have shown the significant antimicrobial activity.

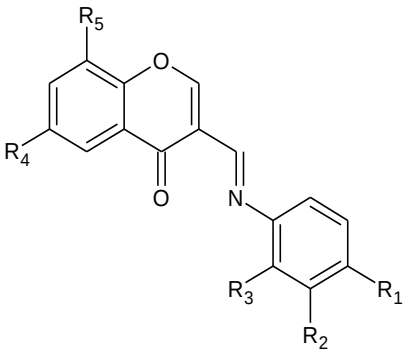
Kavitha et al., synthesized a series of Co(II) complexes of formyl chromone Schiff bases and the ligands and Co(II) complexes were tested against bacterial species *Proteus vulgaris, Staphylococcus aureus, Klebsiella pnuemoniae, Bacillus subtilis and Candida albicans* (fungi).[32]



Co(II) complex of 2-((4-oxo-4H-chromen-3-yl)methylneamino) benzoic acid showed good activity against all bacteria and fungi strains.

Sharad et al., synthesized a series of Schiff bases derived from 3-formyl chromones and various aromatic aniline in aqueous media with microwave as an energy source. The newly synthesized compounds were tested for antimicrobial activity against bacterial strains *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*

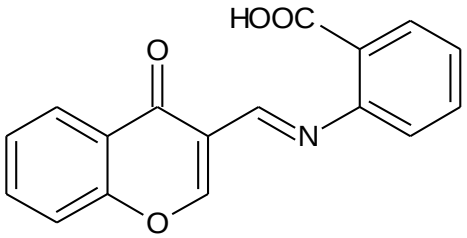
.[33]



	R_1	R_2	R_3	R_4	R_5
a	H	H	H	CH_3	H
b	H	CF_3	H	CH_3	H
c	H	CF_3	H	Cl	Cl
d	Cl	H	CF_3	CH_3	H
e	H	H	CH_3	CH_3	H
f	Cl	H	H	CH_3	H
g	OCH_3	H	H	CH_3	H

Schiff bases showed good to excellent antimicrobial activities against various bacterial and fungal strains.

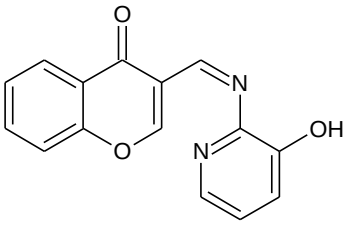
Gyana et al., synthesized a series of Cu(II) , Ni(II) , Co(II) , Mn(II) and Zn(II) complexes from the schiff base ligand 2-[(4-oxo-4H-chromen-3yl) methyleneamino] benzoic acid , which is synthesized by the reaction between chromone-3-carbaldehyde and o-amino benzoic acid. The ligand and its complexes were screened for anti bacterial activity against *E.coli, L.bacillus, B.subtilis and S.aures.*[34]



The metal complexes showed potent anti bacterial activity.

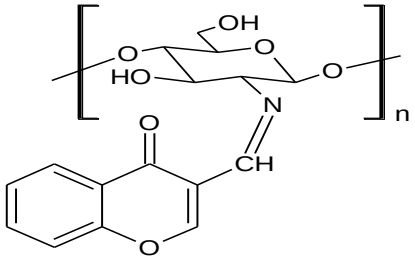
Laxma Reddy et al., synthesized Ni(II) and Zn(II) complexes from tridentate 3-formyl chromone Schiff bases with 2-amino thiophenol, 2-amino phenol, 2- amino benzoic acid and 2-amino 3-hydroxy pyridine. The synthesized ligands and metal complexes were tested

against bacterial species *Proteus vulgaris, Staphylococcus aureus, Klebsiella pnuemoniae, Bacillus subtilis and Candida albicans* (fungi).[35]



Zn(II) complex of 3-((3-hydroxypyridin-2-ylimino)methyl)-4H-chromen-4-one showed good activity against all bacteria and fungi strains.

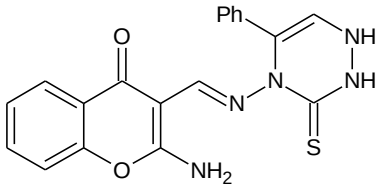
Santosh et al., synthesized chitosan-chromone derivative gels by reacting chitosan with chromone-3-carbaldehyde, followed by solvent exchange, filtration and drying by evaporation. The presence of free reactive amino groups leads to the possibility of forming a Schiff base of chitosan with chromone-3-carbaldehyde. The chitosan-chromone derivative were evaluated for antimicrobial activity against *E.coli*[36]



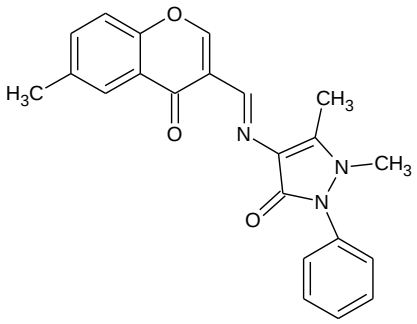
The chitosan-chromone derivative had a dose-dependent antimicrobial activity against *E. coli* .

Ibrahim et al., synthesized some new heterocyclic schiff bases containing 1,2,4-triazole or 1,2,4-triazine derivatives combined with chromone moiety via the reaction of thiocarbohydrazone with some 1,2-

bifunctional electrophiles. The newly synthesized compounds were screened for their antimicrobial activity.[37]

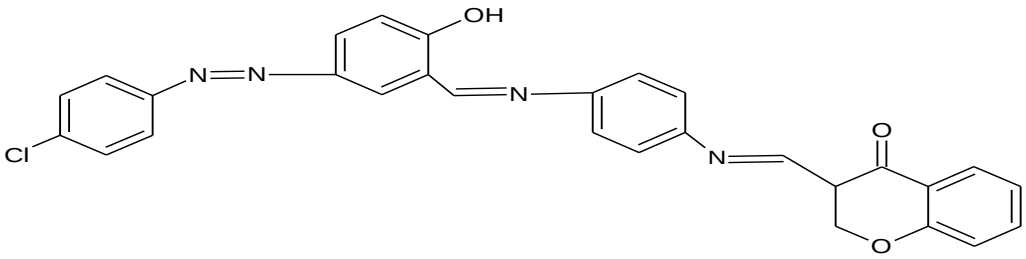


Tudor Rosu et al., synthesized coordination compounds of Cu(II), VO(II), Ni(II), and Mn(II) with the schiff base obtained through the condensation of 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one with 3-formyl-6-methyl-chromone and evaluated the in vitro antibacterial activity against *Staphylococcus aureus , Klebsiella pneumonia* , *Escherichia coli*, and *Pseudomonas aeruginosa*.[38]



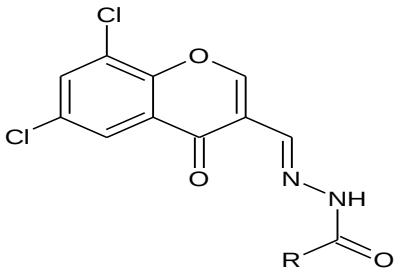
The quantitative antimicrobial activity test results proved that both the ligand and complex combinations have specific antimicrobial activity, depending on the microbial species tested.

C. Anitha et al., synthesized Azo-Schiff-base complexes of VO(II), Co(II), Ni(II), Cu(II) and Zn(II) derived from the condensation of 5-(4-chloro-phenylazo)-2-hydroxy benzaldehyde, 3-formylchromone and p-phenylene diamine. The ligands and their metal complexes were tested for their inhibitory effects on the growth of bacteria *S. aureus, E. coli, S. enteric typhi, B. subtilis* and fungus *C. albicans*.[39]



The Cu(II) and Zn(II) complexes showed greater antibacterial activity and Co(II), Ni(II) and Zn(II) complexes showed greater antifungal activity for *C. albicans.*

Zahid H. Chohan et al., synthesized Co(II) complexes of Schiff bases derived from 3,5-dichloroformylchromone and aryl hydrazide. The ligands and their Cu(II) complexes were screened for their in-vitro antibacterial activity against four Gram-negative *(Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri)* and two Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) bacterial strains.[40]



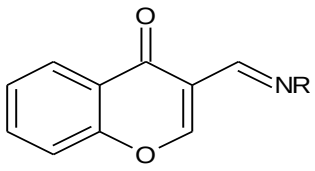
R= C_6H_5OH, C_6H_5Br, C_6H_5Cl, $C_6H_5NH_2$, 

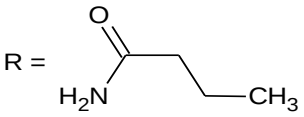
, 

The activity against all the Gram-negative and Gram-positive species was increased by coordination of the ligands with the Cu(II) metal ion.

As anti inflammatory agents

Khalid et al., synthesized a series of Schiff bases of 3 formyl chromone .The synthesized compounds were evaluated for anti inflammatory activity by using various *in vitro* and *in vivo* assay models.[41]

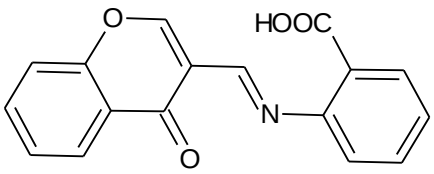




showed the highest activity.

As antioxidants

Kavitha et al., synthesized Pd(II) complexes of 3-formyl chromone schiff bases and the antioxidant activity of Pd(II) complexes were evaluated using DPPH free radical scavenging method.[25]

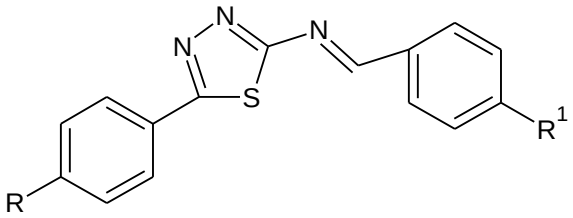


Pd(II) complex of 3-((2 -hydroxyphenylimino) methyl)-4H-chromen-4-one showed activity comparable to standard.

SCHIFF BASES OF 2- AMINO 5 -ARYL 1,3,4 THIADIAZOLE

AS ANALGESIC

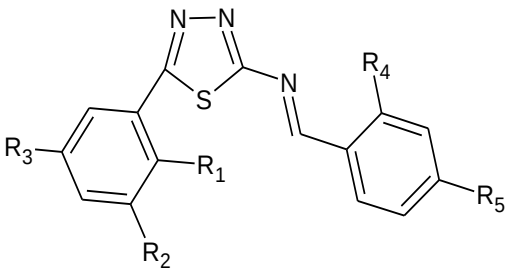
Alok et al., synthesized schiff bases of 2-amino-5-aryl-1,3,4-thiadiazole derivatives with different aromatic aldehyde. All the compounds were evaluated for their analgesic activity against swiss albino mice using hot plate method.[42]



R= OCH_3, OH, Cl, $N(CH_3)_2$; R^1 = OH showed promising analgesic activity.

As anthelmintic

Bijo et al.,synthesized some Schiff bases of 5-phenyl substituted, 2-amino 1, 3, 4 thiadiazole derivates and anthelmintic activity of the synthesized compounds were investigated. Parameters under study were mean paralysis and mean lethal time in Pheretima posthuma.[43]

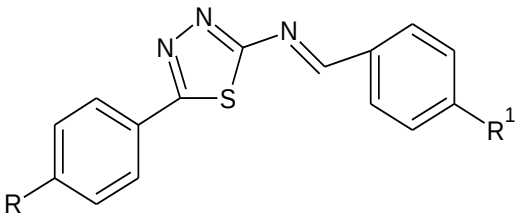


R_1	R_2	R_3	R_4	R_5
OH	H	H	H	Cl
OH	NO_2	NO_2	H	Cl
$OCOCH_3$	H	H	H	Cl
H	H	H	H	H
H	NO_2	H	H	Cl
H	NO_2	H	OH	H
H	NO_2	H	H	H

All the compounds showed significant anthelmintic activity**.**

As anti inflammatory agent

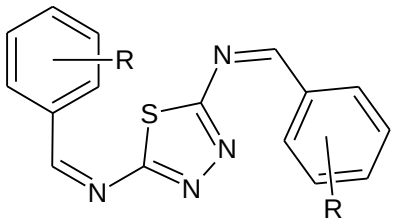
Alok et al., synthesized Schiff bases of 2-amino-5-aryl-1,3,4-thiadiazole derivatives with different aromatic aldehyde and the compounds were evaluated for their anti-inflammatory activity against Wister albino rats by Carrageenan induced paw edema method.[42]



R=OH	R'=OH
R=Cl	R'=OH
R=OCH_3	R'=NO_2
R=$N(CH_3)_2$	R'=NO_2

As antioxidants

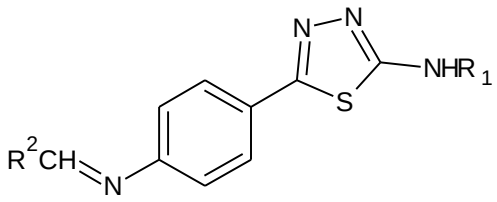
Abbas et al., (2012) synthesized a series of Schiff's bases containing 1,3,4-thiadiazole (2,5-di(N-substitutedbenzylideneamino)- 1,3,4-thiadiazole) and the antioxidant properties were measured using the metal ions (Fe^{+3}, Cu^{+2}), Ferrozine and 2,9-dimethyl-1,10-phenanthroline (neocuproine).[44]



R= 4- OH showed significant activity.

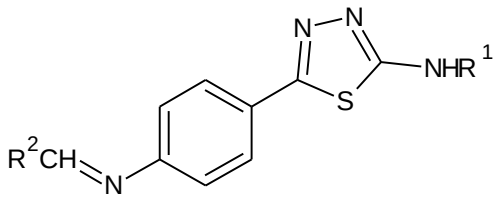
As anti tubercular agents

Sevim et al., synthesized a series of new Schiff bases through the condensation reaction of 1,3,4- thiadiazoles containing an aromatic primary amine and 3-hydroxybenzaldehyde, salicylaldehyde, 5-nitrofurfuraldehyde or 3-nitrobenzaldehyde. The synthesized compounds screened for antituberculosis activity against *Mycobacterium tuberculosis H37Rv* using BACTEC 460 radiometric system.[45]



R^1= Ph ; R^2 = 2- OH Ph showed the highest activity.

Dilmaghani et al., synthesized a series of new Schiff bases by the condensation reaction of 5-(4-aminophenyl)-N-aryl-1,3,4-thiadiazol-2-yl amines with salicylaldehyde, 3-hydroxy benzaldehyde, 4-hydroxy benzaldehyde, 5-bromo salicylaldehyde, 5-chloro salicylaldehyde, 4-methoxy benzaldehyde, 3-nitro benzaldehyde, and 4-nitro benzaldehyde. The synthesized compounds were tested for their antimicrobial efficiency against *Mycobacterium smegmatis PTCC 1307 in vitro.* [46]

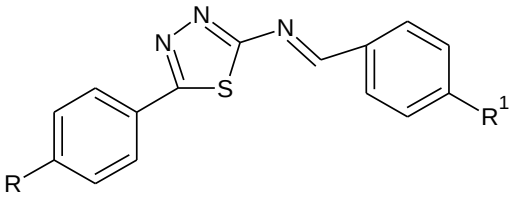


R^1 = 4- BrC_6H_4 ; R^2 = 2- HOC_6H_4 showed highest antiproliferative activity against *M. smegmatis*.

As antimicrobial agents

Alok et al., synthesized Schiff bases of 2-amino-5-aryl-1,3,4-

thiadiazole derivatives with different aromatic aldehyde and the compounds were evaluated for antibacterial activity.[47]

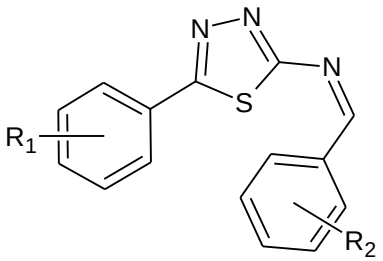


R=OH	R'=OH
$R=NO_2$	R'=OH
$R=OCH_3$	R'=OH
$R= N(CH_3)_2$	$R'= NO_2$

The compounds showed significant antibacterial activity against *Staphylococcus aureus* (gram-positive bacteria) *and E. coli* (gram-negative bacteria).

Abdalla M. Khedr et al., reported the synthesis *of* new mono- and binuclear copper(II) complexes with Schiff bases derived from the condensation of 2-amino-5-substituted-aryl-1,3,4-thiadiazole with substituted aryl aldehydes .

The synthesized complexes and ligands were screened *in-vitro* for their antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli*) and fungi (*Aspergillus flavus* and *Candida albicans*).[48]



R_1=H, 4-Cl, 2-NO_2, 4-OH, 4-$N(CH_3)_2$, 2-OH

R_2=4-NO_2 , C_6H_5N , 2-Cl ,4-OCH_3 , C $_6H_5N$,2-OH.

CHEMISTRY

INTRODUCTION[49]

Chromones (4H- Benzopyran- 4-ones) are the heterocyclic compounds containing benzopyron network with substituted keto group on pyron ring. It is an isomer of coumarin.

3-Formylchromone (4-oxo-4H-chromene-3-carbaldehyde), is a fairly versatile molecule that is easily synthesized and frequently used for the incorporation of chromone moiety into other aromatic/heterocyclic molecules, or to construct new scaffolds based on this molecule.

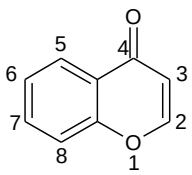
3-Formylchromones occupy a unique position due to three reasons:

i) They are carrying significant biological activities like anticancer, antimicrobial, antioxidant, anti H.pylori activity, urease inhibitory activity.

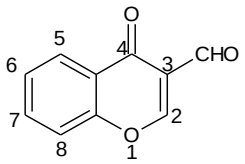
ii) They have comfortable preparation by Vilsmeier-Haack reaction in very good yields.

iii) They are attractive intermediates.

Much of the synthetic utility of 3-formyl chromones is due to the presence of three electron deficient centers, the carbonyl carbon atoms C-4 and C-3' of the keto of formyl groups respectively, and carbon atom C-2 of the chromone ring. Formyl chromones have been of synthetic and biological interest because of their synthetic utility based on the three electrophilic carbon atoms and the biological activities associated with the formyl chromone derivatives or structurally modified compounds.



4H- Benzopyran- 4-ones

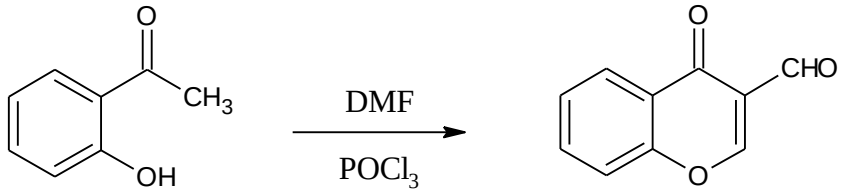


4-oxo-4H-chromene-3-carbaldehyde

GENERAL METHODS OF SYNTHESIS OF 3-FORMYL CHROMONE[50,51]

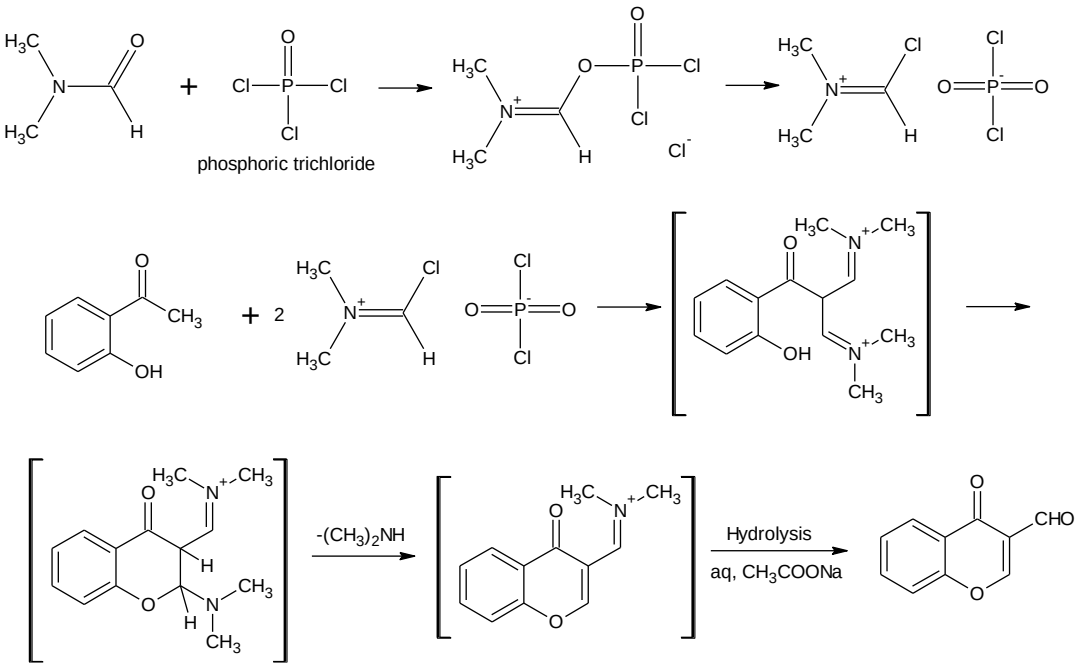
I) Vilsmeier Haack Reaction.

The reaction of acetophenone with substituted amide, typically with N,N dimethyl formamide (DMF) and phosphorous oxy chloride ($POCl_3$) leads to the formation of 3 -formyl chromones.



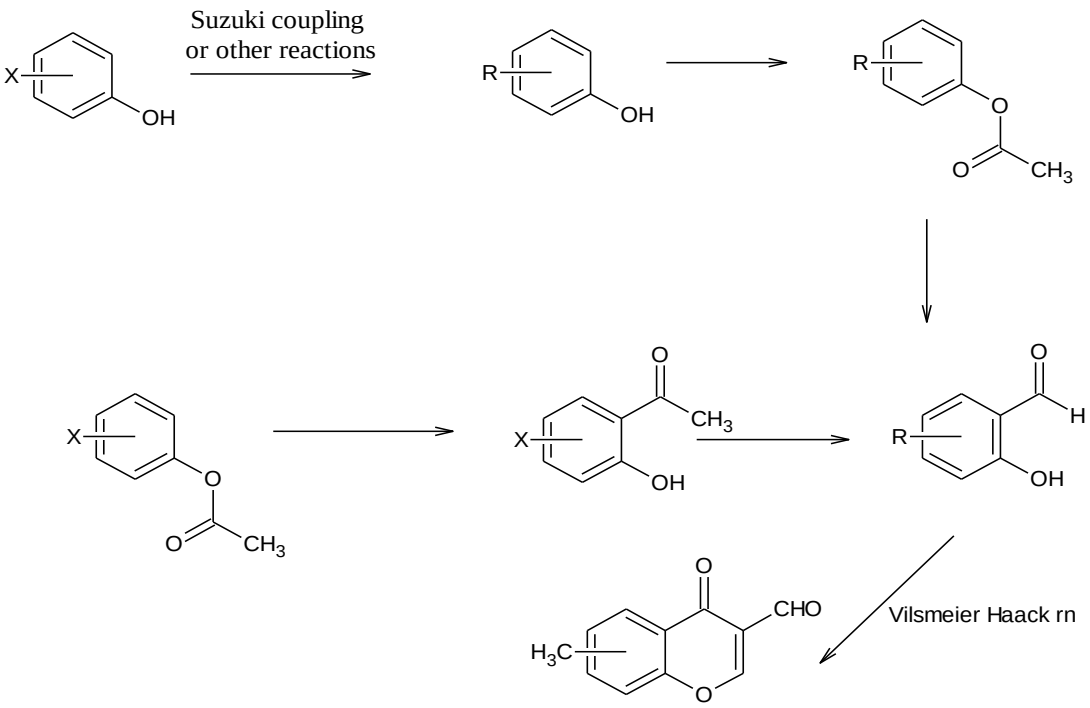
Acetophenone 3- formyl chromone

MECHANISM



The active reagent is substituted chloro iminium ion that is formed by the reaction of DMF and $POCl_3$. This chloro iminium ion then attacks the electron rich arene to give the corresponding aromatic ketone or aldehyde.

II) One method for synthesis of 3 formyl chromone involves the preparation of appropriately substituted phenol derivatives followed by Friedel-Crafts acylation to obtain 2-acetylphenol derivatives. Alternatively, 2-acetylphenol derivatives could be obtained by acetylation prior to the introduction of the substituent R. The core structure was then formed by Vilsmeier-Haack reaction in which 2-acetyl phenol moiety in 2-acetylphenol derivative was converted to 3-formylchromone structure in the presence of $POCl_3$ and DMF.

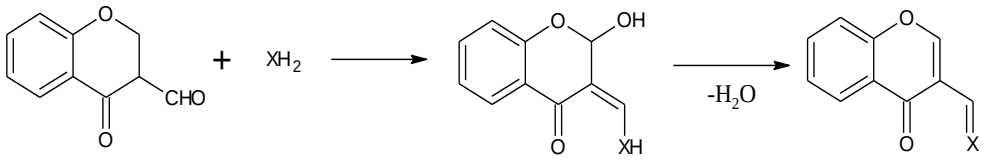


REACTIONS OF 3- FORMYL CHROMONE[52- 57]

3- formyl chromone possesses an endocyclic olefinic bond, an α,β-unsaturated carbonyl functionality, three electrophilic centres (C-2, aldehydic and ketonic carbons). These unique features make the chromone amenable to various reactions.

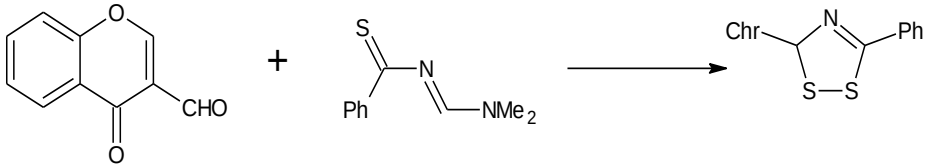
1. Nucleophilic Addition

3-Formylchromone is a good Michael acceptor towards most, if not all, nucleophiles. Thus, a nucleophile XH_2 such as an amine, hydrazine, monosubstituted hydrazine, hydroxylamine or an active methyl or methylene compound in conjugation with an appropriate base undergoes Michael addition with concomitant opening of the pyran ring and subsequent recyclization. (i.e. domino Michael–retro-Michael–Intramolecular 1,2-addition) giving the hemiacetal that leads to final compound by water elimination.



a) Addition of sulfur nucleophiles

3-Formylchromone when heated with 2-phenyl-4-dimethylamino-1-thia-3-azabutadiene in a sealed tube gives1,2,4-dithiazole.

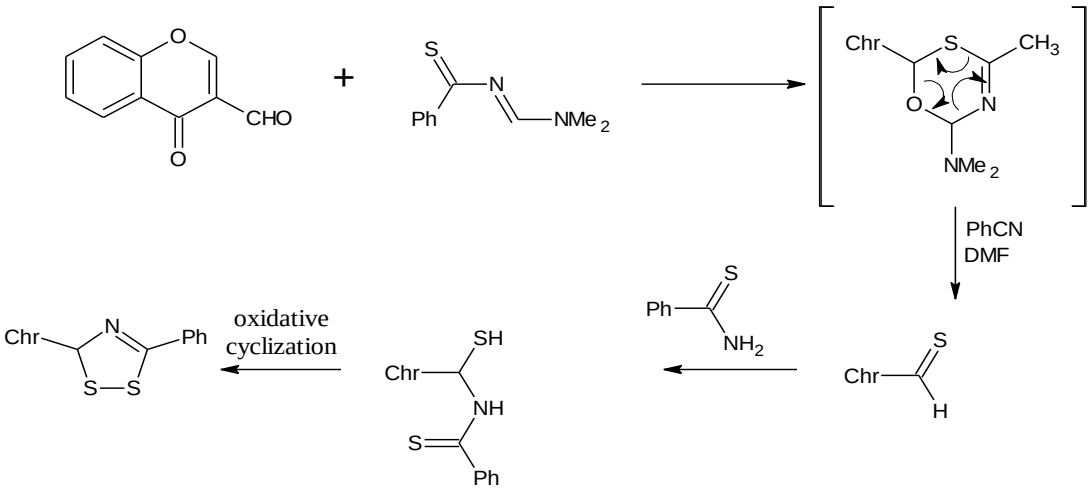


MECHANISM

The first step involves thionation of 3- formyl chromone to thioformyl

chromone through the [4+2] cycloadduct of 3- formyl chromone and thiazadiene.

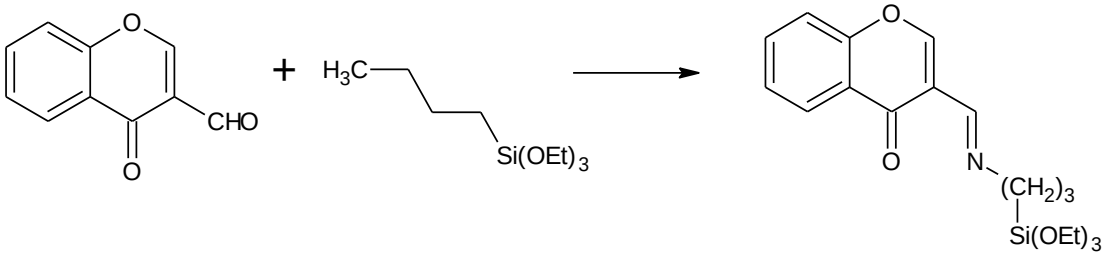
The second step involves the reaction of 3- thioformyl chromone with a molecule of thiobenzamide followed by oxidative cyclisation.



b) Addition of nitrogen nucleophiles

i) Addition of aliphatic amines.

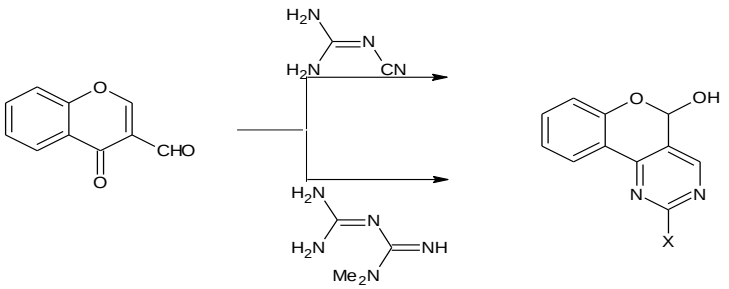
Schiff bases are obtained when ChrCHO is treated with an aliphatic or aromatic amine RNH_2.



iii) Reaction with guanidine.

Reaction of 3-formyl chromone with cyano guanidine or metformine

gives biologically important pyrimidine **.**

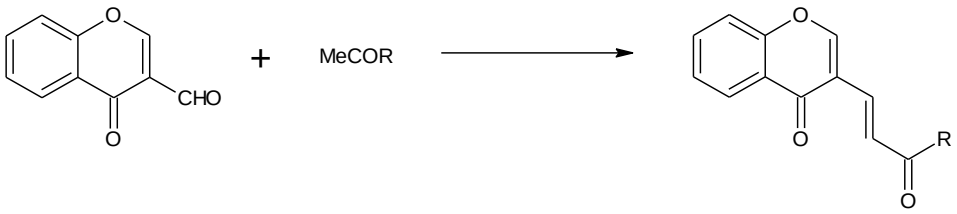


X= NHCN, NHC(=NH)NMe_2

c) Addition of carbon nucleophiles

i) Addition of active methyl and acyclic methylene compounds.

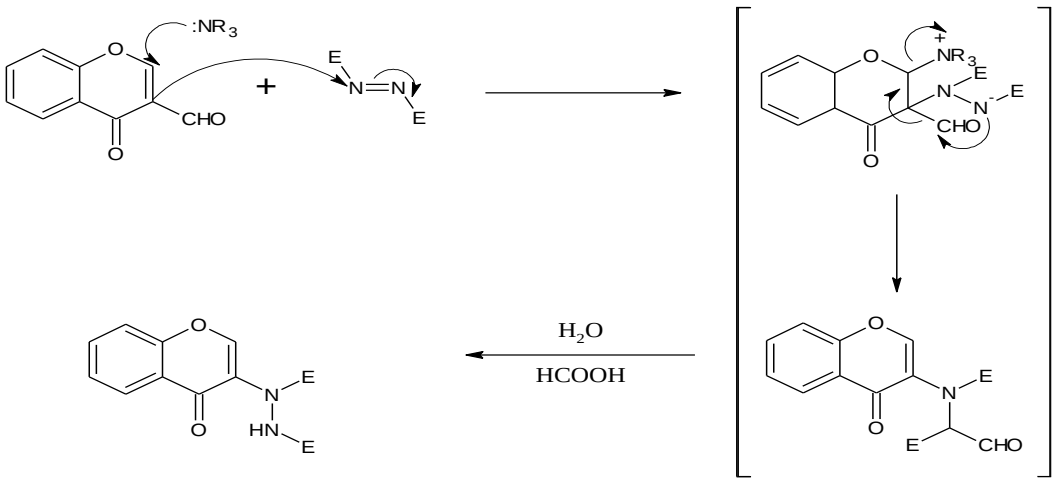
The aryl (or hetaryl) methyl ketone condenses with 3-formylchromone under various conditions to give the chalcones.



R= Aryl, hetaryl

2) Baylis-Hillman reaction

Synthesis of 3-hydrazino chromone from 3 formyl chromone and azidodicarboxylate (E = CO_2Et or CO_2Me) in the presence of DABCO (here written as NR_3) involves an aza-Baylis-Hillman type reaction followed by deformylation.

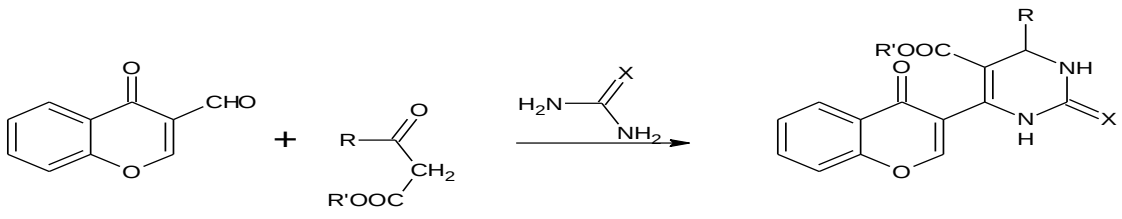


4) 3-Formylchromone as a Component in One Pot Multi component Synthesis

Here 3-formylchromone with at least two other different reactants, if not more, put together at a time in one reaction vessel. As ChrCHO contains three electropositive centres, most of the other reacting partners should function as nucleophiles either in the absence or in the presence of a suitable catalyst.

Biginelli Reaction

ChrCHO when subjected to Biginelli reaction with a β-ketoester and guanidine or urea or thiourea behaves as a simple aldehyde to give the 1,4-dihydropyrimidine derivative .

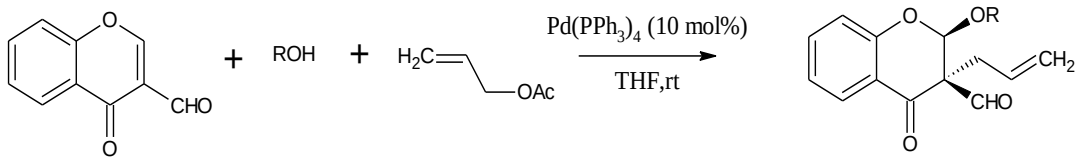


R = Me, R^1 = Et; X = O,S,NH

5) Three component reactions of 3-formylchromone with reagents

other than a nitrogen Nucleophile.

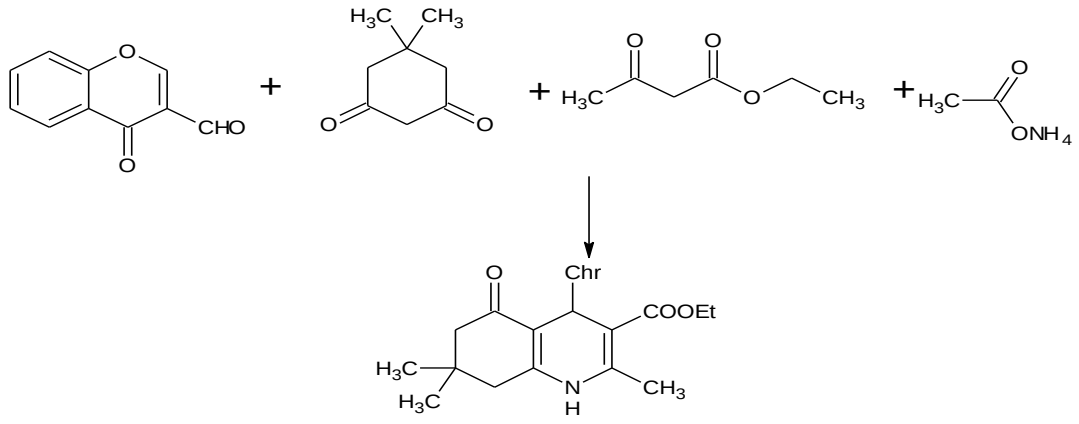
Palladium catalyzed three component coupling reaction between 3 formylchromone, alcohol and allyl acetate leads to the highly substituted chromanone.



6) 3-Formylchromone as a component in the four component reactions

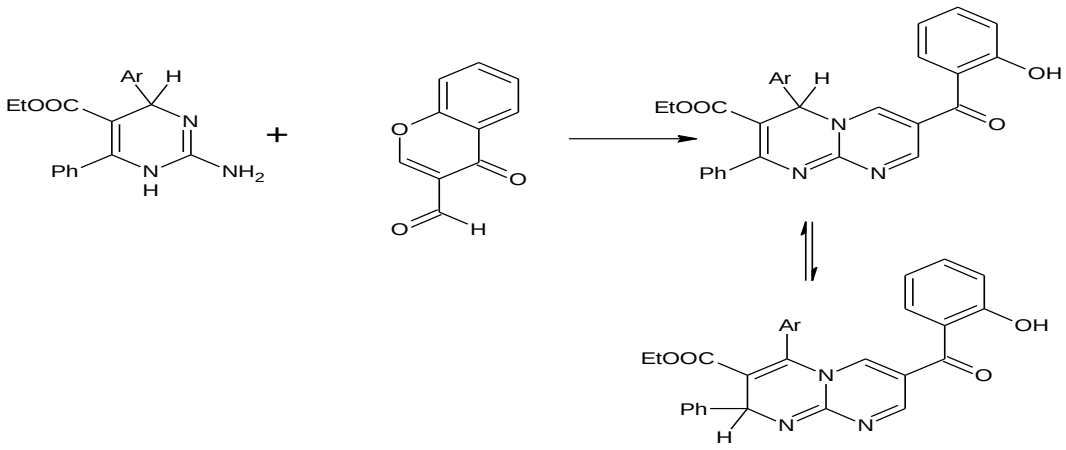
Hantzsch synthesis

Application of the Hantzsch procedure for synthesis of 1,4-dihydropyridine in one-pot reaction of ChrCHO, dimedone, ethyl acetoacetate and ammonium acetate gives the cyclohexanopyridine derivative.



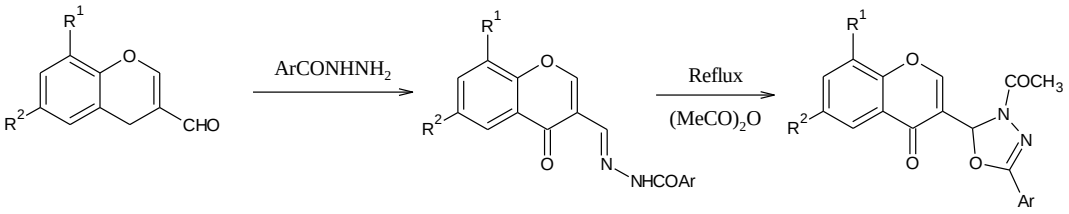
7. Reaction of 3- formyl chromone to yield Pyrimido[1,2-a]pyrimidines .

Pyrimido[1,2-a]pyrimidines were prepared from dihydro amino pyrimidines and chromone-3- aldehydes . Reactions are much faster and better yields have been obtained with microwaves.



Ar= C_6H_5,4- $CH_3C_6H_4$, 4-ClC_6H_4

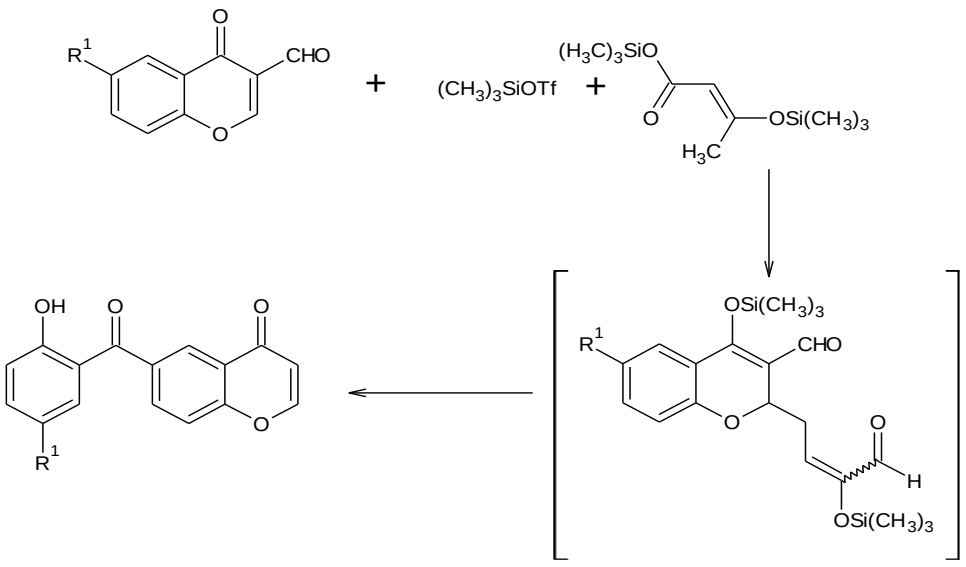
8. Reaction of 3- formyl chromone to yield 1,3,4- oxadiazol- 2 chromone The treatment of the 3-formylchromones with aroylhydrazines give the corresponding aroylhydrazones which in the presence of acetic anhydride undergoes heterocyclization to afford 3-(3-acetyl-5-aryl-2,3-dihydro-1,3,4-oxadiazol-2-yl) chromones .



Michael–Retro-Michael–Aldol Reaction

The reaction of 3-formylchromones with Me $_3$SiOTf and 1,3-bis(silyl

enol ether)afforded the 4-(2-hydroxybenzoyl)phenols . The formation of the products could be explained by a domino ‘‘Michael–retro-Michael–Aldol’’ reaction.

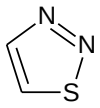


R^1= H, alkyl, aryl

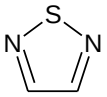
2- AMINO 5 -ARYL 1,3,4 THIADIAZOLES[58-60]

INTRODUCTION

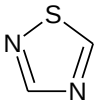
Thiadiazole is a 5-membered ring system containing hydrogen-binding domain, sulphur atom, and two-electron donor nitrogen system (–N=C–S) that exhibit a wide variety of biological activity. They occur in four isomeric forms in the nature viz. 1,2,3-thiadiazole ; 1,2,5-thiadiazole ; 1,2,4-thiadiazole ; and 1,3,4- thiadiazole .



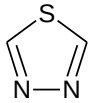
1,2,3- thiadiazole



1,2,5- thiadiazole



1,2,4- thiadiazole



1,3,4- thiadiazole

Four Isomeric Forms of Thiadiazole

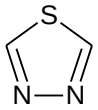
Among these four types of thiadiazole, 1, 3, 4- thiadiazole is well known.

1,3,4-Thiadiazoles were conveniently divided into three subclasses:

a) Aromatic systems which include the neutral thiadiazole (4).

b) Mesoionic systems (4a) which is defined as five-membered heterocycles which are not covalent or polar and possess a sextet of electrons in association with the five atoms comprising the ring.

c) Non aromatic systems such as the 1,3,4-thiadiazolines (4b, 4c) and the tetrahydo 1,3,4-thiadiazolidines (4d).



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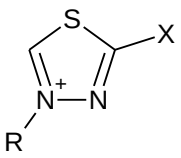
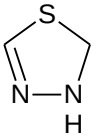
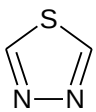


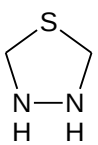
(4a)



(4b)



(4c)



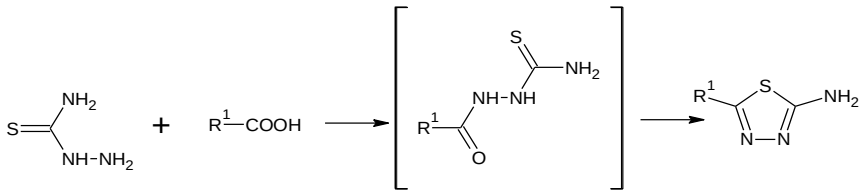
(4d)

Literature survey revealed that various thiadiazoles posses pharmacological activities including antitumor , antiviral, antibacterial, amoebicidal, antiinflammatory, antitubercular, antipyretic,CNS depressant, herbicidal , insecticidal, pesticidal and hypoglycemic.

GENERAL METHODS OF SYNTHESIS OF 2 AMINO 5 ARYL 1,3,4 THIADIAZOLE

1) From Thiosemicarbazides and carboxylic acid derivatives:

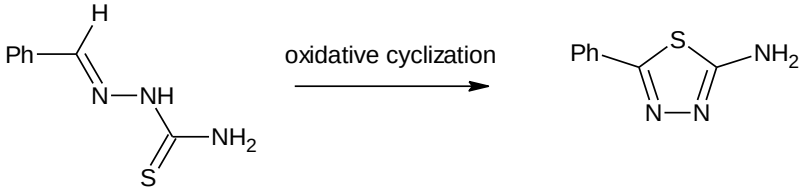
i) Reaction of thiosemicarbazide with carbon source reagents in the presence of dehydrating agents provides a useful route to 1,3,4-thiadiazoles . The reaction proceeds via the monothiodiacyl hydrazines. Carbon source may be aliphatic, aromatic, heterocyclic carboxylic acids, esters or acid chlorides and the dehydrating agents are phosphorus oxychloride , sulfuric acid , and PPA



R^1= phenyl, isopropyl, benzofur-2-yl, methyl

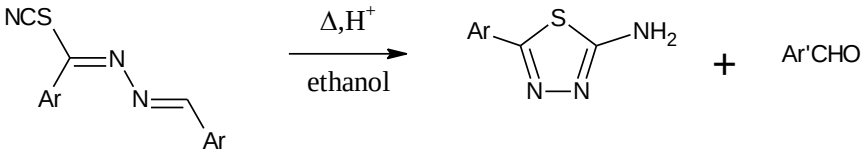
2) From thioacylhydrazone

Oxidative cyclization of thioacylhydrazone by common oxidants such as bromine , ferric chloride , ammonium ferric sulfate or potassium permanganate provided 2-amino-5-phenyl-1,3,4-thiadiazole .



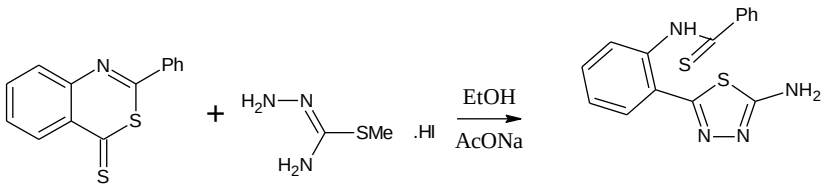
3) From hydrazonoyl thiocyanate

When hydrazonoyl thiocyanates were heated in toluene, they were converted into the thiadiazole derivatives, but on acid hydrolysis in refluxing ethanol it will give aldehyde and the aminothiadiazole derivatives.



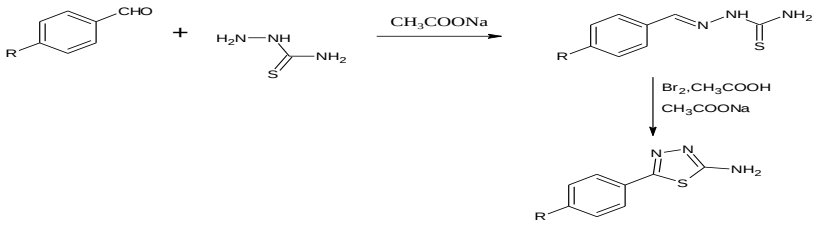
4) From benzothiazines

4*H*-3,1-Benzothiazine-4-thione was treated with S-methyl isothiosemicarbazide hydroioide in ethanol containing sodium acetate to yield the 5-aryl-2-amino-1,3,4-thiadiazole . A suitable mechanism for this transformation requires the addition of one of the amino groups from the thiosemicarbazide to the thiocabonyl group of the benzothiazin-4-thione followed by ring opening and recyclization with elimination of CH_3SH to give the thiadiazole .



5) From thiosemicarbazide and aldehydes

2-amino-5-aryl-1,3,4-thiadiazole can be prepared by the reaction of thiosemicarbazide, sodium acetate and aromatic aldehyde.

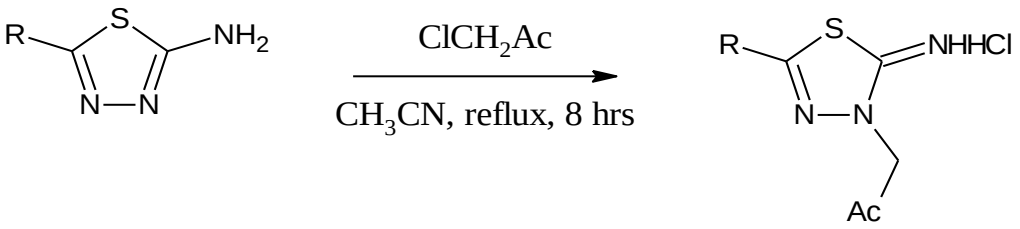


REACTIONS OF 2- AMINO 5- ARYL 1,3,4 THIADIAZOLE

I. Reactivity of 1,3,4-thiadiazole ring

1. Electrophilic attack at nitrogen

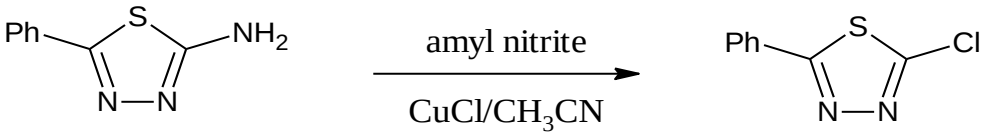
The ring nitrogens react with electrophiles to afford either 1,3,4-thiadiazolium salts or 1,3,4-thiadiazol-2(3*H*)-ones. While *N*-alkylation is the most common electrophilic reaction of 1,3,4-thiadiazole, reactions with acyl and cyanogen halides as well as Mannich salts have also been reported. 2-Amino-1,3,4-thiadiazole reacts with chloroacetone to give the *N*- alkylated thiadiazolimine.



2. Nucleophilic attack at carbon

Nucleophilic reactions at the carbon atoms of 1,3,4-thiadiazoles occur readily owing to the electron-deficient nature of the ring.

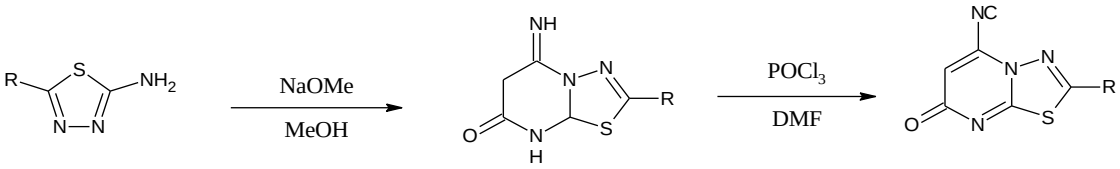
2-Amino-1,3,4-thiadiazoles undergo Sandmeyer reactions to afford 2-halo-1,3,4-thiadiazoles. Diazotization followed by a Sandmeyer reaction of the 2-amino-5-phenyl-1,3,4-thiadiazole with CuCl generated *in situ* will give 2-chloro-5-phenyl-l,3,4-thiadiazole.



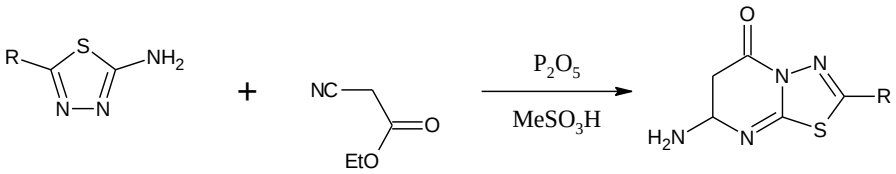
II) Reactivity of substituents attached to ring carbon atoms

Nitrogen substituents:

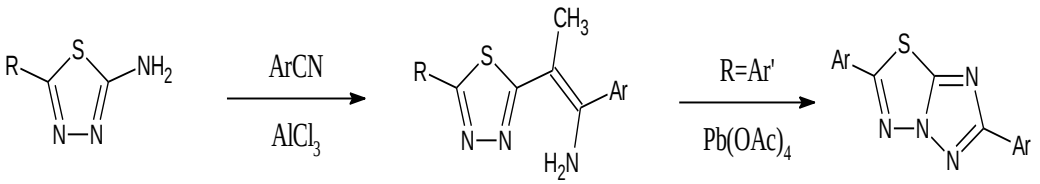
- Treatment of 2-amino-5-substituted thiadiazoles with ethyl cyanoacetate in the presence of sodium methoxide gave the 2-substituted- 5- imino-6*H*-[1,3,4-thiadiazolo[3,2-a]pyrimidine-7-one which on further treatment with DMF/$POCl_3$ yielded the 5-isocyanide derivative **.**



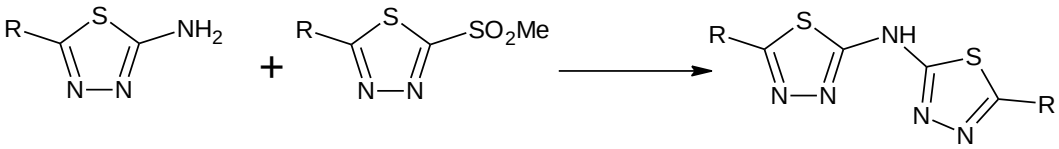
- Treatment of 2-amino-5-substituted thiadiazoles with ethyl cyanoacetate in the presence of P_2O_5 and CH_3SO_3H , 7-amino[1,3,4]-thiadiazolo[3,2-a]pyrimidine-5-one was obtained.



- Reaction of 2-amino-5-aryl-thiadiazole with aryl nitriles in the presence of aluminum chloride produced the aryl amidine which was oxidized with lead tetraacetate to yield 2,6-diaryl-[1,2,4]-triazolo[5,1-b]- 1,3,4-thiadiazoles . The yields of amidines depend on the reactivity of the nitriles. Decreasing the electron density of the cyano group by such electron-withdrawing groups as *p*-nitrophenyl, and 2 and 4-cyanopyridyl led to higher yields as compared to unsubstituted benzonitrile.

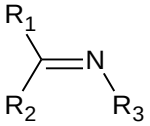


- A bis thiadiazole can be prepared by reacting the sodium salt of 2-amino-5-aryl-thiadiazole with 2-methane sulfonyl-5-*t*-butyl-1,3,4-thiadiazole.



SCHIFF'S BASE[61, 62]

INTRODUCTION



R_1, R_2= alkyl,aryl or H

R_3= alkyl ,aryl

General structure of a Schiff base

Schiff bases are biologically as well as synthetically important nitrogen containing compounds having azomethine group (-CH=N-) and are formed by condensation between primary amines and carbonyl compounds.Schiff bases exhibits a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral,anti-tubercular and antipyretic properties

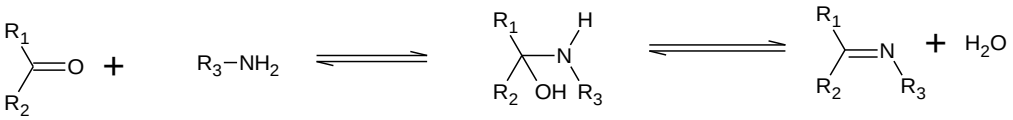
Schiff bases have a large number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the carbon-nitrogen double bond. Reactions of this type have been put to good use in natural product synthesis.

Schiff bases appear to be an important intermediate in a number of enzymatic reactions involving interaction of an enzyme with an amino or a carbonyl group of the substrate. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation of a primary amine in an enzyme usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base.

GENERAL METHODS FOR THE FORMATION OF C=N BONDS

a) Amine carbonyl condensations:

The classic method for the introduction of the carbon nitrogen double bond into the molecule involves the condensation of aldehydes and ketones with a variety of amino compounds (amines, hydroxylamines, hydrazines) followed by elimination of elements of water to give corresponding azomethines.

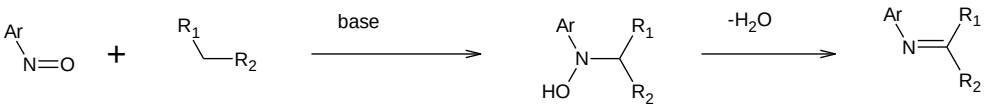


R_1,R_2= H, alkyl,aryl

R_3= alkyl, aryl, OH,OR,NHR

b) Condensation reaction involving active methylene compounds:

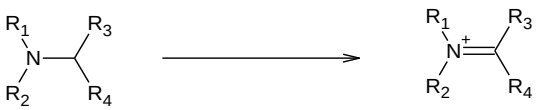
Aromatic nitroso compounds undergo base catalysed condensation with active methylene compounds to give intermediate adducts (hydroxylamine derivatives) which can be dehydrated to azomethines.



c) Dehydrogenation (oxidation) of amino compounds to azomethines:

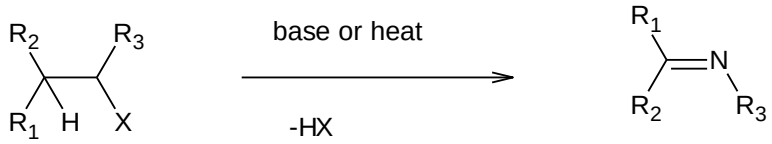
The dehydrogenation of primary or secondary alkyl amines over nickel, platinum, chromium catalysts or in contact with sulphur, or selenium gives acceptable yields of corresponding azomethines.

Reagent used: Hydride transfer reagents (diazonium fluoroborates, trityl perchlorate)



d) Elimination reaction leading to azomethines:

Thermal and base catalysed elimination of substrates derived by electroplilic attack (halogenations, nitrosation, nitration, sulphonation) at the nitrogen atom of primary and secondary alkylamines.



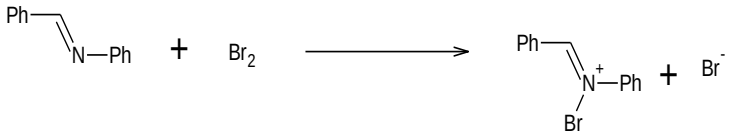
GENERAL REACTIONS OF SCHIFF BASES

a) Reaction with electrophiles

Electropliles attack predominantly at nitrogen atom in the azomethines.

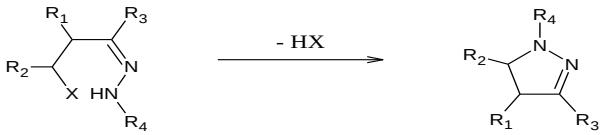
i) Halogenation

Halogens are reported to add in a 1,2-fashion to the carbon-nitrogen double bond in N-arylaldimines. The product of the reaction of benzylidene aniline with bromine in carbon tetrachloride is formulated as an N-bromoiminium bromide.



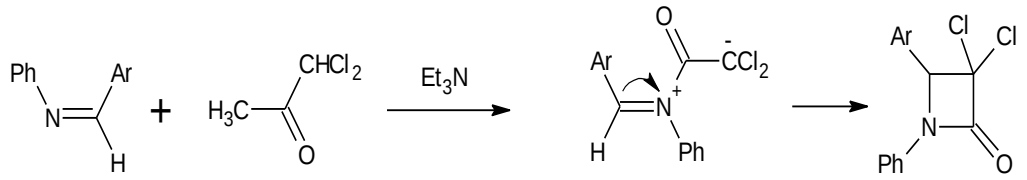
ii) Alkylation

Direct alkylation of N-alkyl aldimines and ketimines occurs at the nitrogen atom to give corresponding iminimum salt. N-alkylation of N-monosubstituted hydrazones has been applied intramolecularly providing a general route to pyrazolines.



iii) Acylation:

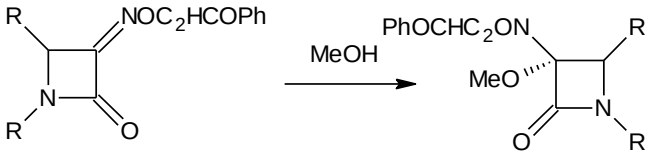
Acylation of Schiff's base by acid anhydrides, acid chlorides and acyl cyanides is initated by attack at the nitrogen atom and leads to net addition of acylating agent to the carbon nitrogen double bond.



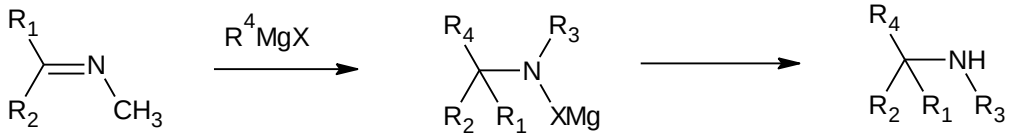
b) Reaction with nucleophiles

Nucleophilic reagents attack azomethines at imidyl carbon atom.

1) Alkoxide adds to Schiff's base giving corresponding α- alkoxyamino compounds.

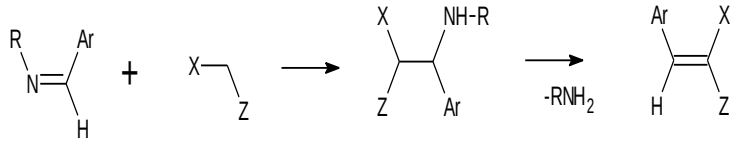


2) Reaction with Grignard reagents: Schiff base lacking hydrogen atoms α to the carbon nitrogen double bond react with Grignard reagents to give adducts which on hydrolytic workup afford secondary amine in excellent yield.



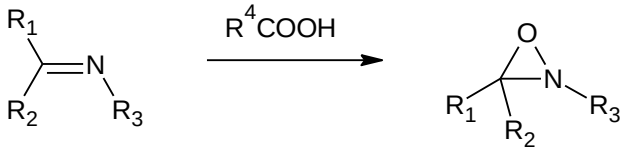
3) Reaction with active methylene compounds: Schiff base react

readily with active methylene compounds to give adducts which tend to eliminate the elements of an amine affording the corresponding alkene.



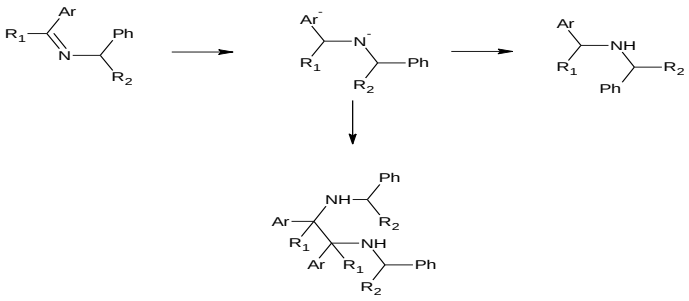
c) Oxidation

Oxidation of Schiff base with a peroxy acid results in cleavage of carbon nitrogen bond to give a carbonyl compound and a nitroso compound. On the other hand oxidation using peroxy acid at low temperature (0 ^{0}C) affords an excellent synthetic route to oxaziridines.



d) Reduction

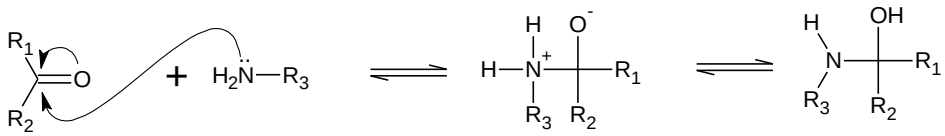
Alkali metals in inert solvents such as ether or toluene tend to promote reductive dimerization by a radical coupling mechanism to afford diamino compound as a major product. Metal proton reagents (sodium, sodium amalgam, magnesium, aluminium in ethanol etc.) smoothly reduce Schiff base to corresponding amines.



MECHANISM

Imines are prepared by a reaction between a carbonyl compound and a primary amine. If the imine contains a hydrogen atom, it is unstable and usually cannot be isolated. However when the imine contains aromatic group on the nitrogen, the resulting imine is stable and can be isolated. The products are called Schiff base.

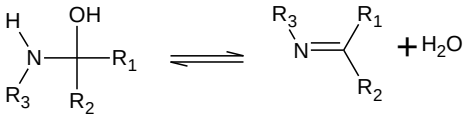
Step 1:



Nucleophilic addition of the amine to the carbonyl compound followed by transfer of a proton from nitrogen to oxygen leads to the formation of tetrahedral carbinolamine intermediate.

Step 2:

Elimination of water to gives the corresponding azomethines.



PURPOSE AND PLAN OF WORK

Enzyme inhibitors are used as potent therapeutic agents for the treatment of various diseases. More than 100 drugs used worldwide are enzyme inhibitors.

Thymidine phosphorylase is a highly expressed protein in many solid human tumours. This has been identified as a potential target in the development of anti-cancer drugs. Abnormally the level of thymidine phosphorylase is highly expressed in various pathological disorders including pancreatic, gastric carcinoma, colon carcinoma, uterine sarcoma, renal carcinoma, breast and lung cancers, astrocytic tumors, cervical intraepithelial neoplasia, carcinomas of the ovary and bladder, Kaposi sarcoma, atherosclerosis, and in various inflammatory diseases. Identification of effective inhibitors of thymidine phosphorylase is, therefore, a need for the treatment of various types of neoplastic and non-neoplastic diseases.

Currently only one TP inhibitor (tipiracil), in combination of trifluridine (a cytotoxin), is recently approved by U.S. FDA for clinical use for the treatment of metastatic colorectal cancer. This combination drug (trifluridine/ tipiracil), marketed as Lonsurf, is also associated with severe side effects, such as myelosuppression, anemia, and neutropenia. Therefore there is need to develop new TP inhibitory agents.

Indeed, a potent inhibitor of TP, 5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]uracil (TPI) entered phase II clinical trials in combination with 5-trifluoromethyl- 2α-deoxyuridine as an orally available anticancer treatment under the name of TAS-102.

3-Formylchromone is a fairly versatile molecule that is easily synthesized and its Schiff bases are associated with a wide range of biological activities including antimicrobial, anticancer, anti inflammatory, antioxidant activities. In addition literature review also revealed that Schiff

bases of 3 formyl chromone exhibit thymidine phosphorylase inhibitory activity.

Thiadiazole is also a potent nucleus which exhibits pharmacological activities such as antitumor, antiviral, antibacterial, amoebicidal, antiinflammatory, antitubercular, antipyretic, CNS depressant and hypoglycemic activities.

Based on the above facts, the present work is focused on synthesis of Schiff bases of 7- hydroxyl - 3 formyl chromone using various 2- amino 5 aryl 1,3, 4 thiadiazole as novel thymidine phosphorylase inhibitors.

3- formyl chromone, 1,3,4 thiadiazole and Schiff bases have been reported to possess antimicrobial properties. Hence the synthesized compounds are screened for their *invitro* antimicrobial activity against selected strains of Gram positive and Gram negative bacteria and fungi.

The present work consists of the following different stages:-

Phase I : Literature review

Phase II : Synthesis of various Schiff bases of 7- hydroxyl 3- formyl chromone.

Phase III : Spectral studies of synthesized compounds

Phase IV : Evaluation of *invitro* Thymidine phosphorylase inhibitory activity.

Phase V : Antimicrobial screening of synthesized compound

Antibacterial studies

Minimum Inhibitory Concentration

Antifungal studies

EXPERIMENTAL WORK

a. MATERIALS AND METHODS

Chemicals used

2,4–dihydroxyacetophenone, dimethyl formamide,phosphorous oxy chloride,thiosemicarbazide, conc. sulphuric acid,2- chloro benzoic acid, 3-chloro benzoic acid, 4- chloro benzoic acid, 2,4- dichloro benzoic acid, 2-nitro benzoic acid, 3- nitro benzoic acid, 4- nitro benzoic acid, 3,5- dinitro benzoic acid, methanol.

Apparatus used

Beakers, test tubes, conical flask, round bottom flasks, reflux condenser, thermometer, glass rods, funnel, mechanical stirrer.

Analytical works

- Melting points were determined using melting point apparatus LAB INDIA MR- VIS Scientific.
- Reactions were monitored by the thin layer chromatography (TLC) on precoated silica gel G plates using iodine vapour as visualizing agent.
- IR spectra were recorded on JASCO FT/IR -140 spectrophotometer in the Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.
- UV spectra were recorded on JASCO V-530 UV/VIS spectrophotometer in the Department of Pharmaceutical analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.
- PMR spectra were recorded using BRUKER AV III FT-NMR 500 MHz in the SAIF, IIT Madras.
- Mass spectra were recorded using JEOL GC Mate GC-MS spectrometer in the SAIF, IIT Madras.

SCHEME

Method

Step 1:Synthesis of 7 hydroxy 3 formyl chromen-4-one[31]

In dry DMF (60 ml) in three neck flask, $POCl_3$ (37.5 ml) was added slowly with vigorous stirring at 50°C. Heating and stirring was continued for 2 hrs at 45-55°C. The solution of resacetophenone (9.12 gm) in DMF (12.5 ml) was then slowly added with stirring at 50°C and stirring was continued for 2 hrs. After cooling the mixture was kept overnight at room temperature and diluted slowly by adding ice cold water (250 ml) and was stirred again for 6hrs. The red crystalline product separated was filtered and recrystallised from methanol.

Step 2: Synthesis of 2-amino-5-aryl-1,3,4- thiadiazole[63]

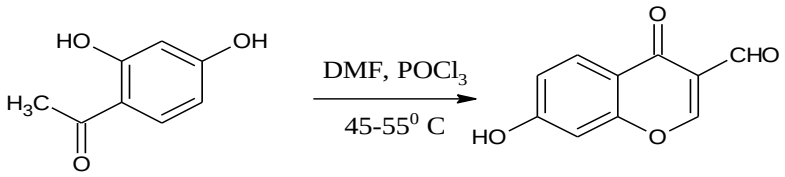
Thiosemicarbazide (0.01 mole), aryl carboxylic acid (0.01 mole) and conc. sulphuric acid (5 ml) were refluxed for 2 hours. The reflux was continued for further time of span until we get a distinct single point on TLC. This hot mixture was poured on to crushed ice. The solid separated was filtered out and washed with water. It was recrystallized from methanol.

Step 3:Synthesis of Schiff bases[31]

Schiff bases were prepared by reaction of equimoles of 7-hydroxy-3-formyl chromen-4-one and various amines. 7-Hydroxy-3-formyl chromen- 4-one (0.01 mole) was dissolved in 5 ml methanol. Amine (0.01 mole) was added with constant stirring. To the resulting mixture 2-4 drops of concentrated H_2SO_4 was added and the mixture was refluxed for 1-2 hrs. After completion of reaction, mixture was poured over crushed ice with stirring. The product obtained was filtered and recrystallised from methanol.

SCHEME

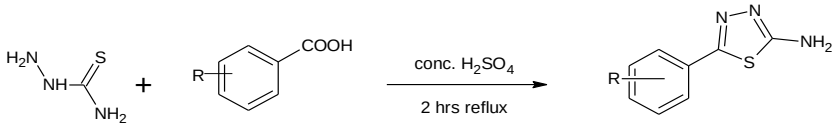
Step 1



2,4 - dihydroxy acetophenone

7 -hydroxy 3- formyl chromone

Step II

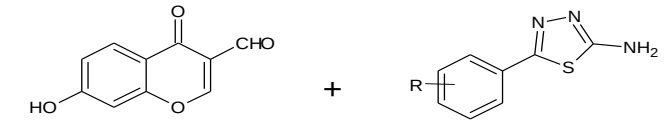


Thiosemicarbazide

Substituted aromatic acid

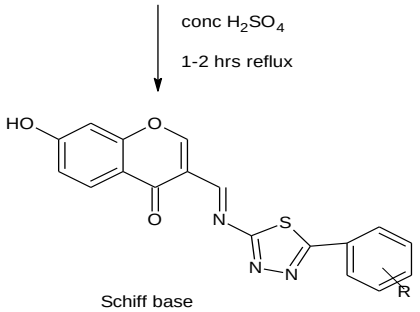
2 - amino 5 - aryl 1,3,4 - thiadiazole

Step III



7 - hydroxy 3 - formyl chromone

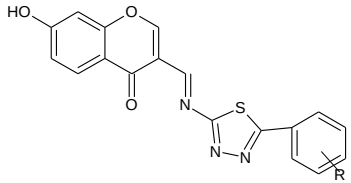
2 - amino 5 - aryl 1,3,4 - thiadiazole



Schiff base

Schiff Base	
Compound code	R
Ia	2-Cl
Ib	3-Cl
Ic	4-Cl
Id	2,4-Cl
Ie	2- NO_2
If	3- NO_2
Ig	4- NO_2
Ih	3,5- NO_2

Table No:1 PHYSICAL CHARACTERISATION OF NEWLY SYNTHESIZED COMPOUNDS



Compound code	R	Molecular formula	Molecular weight	Melting point °C	R_f value	% yield
Ia	2-Cl	$C_{18}H_{10}ClN_3O_3S$	383.80	138.6	0.3617	77%
Ib	3-Cl	$C_{18}H_{10}ClN_3O_3S$	383.80	145.4	0.3409	74%
Ic	4-Cl	$C_{18}H_{10}ClN_3O_3S$	383.80	143.5	0.7272	77%
Id	2,4-Cl	$C_{18}H_9Cl_2N_3O_3S$	418.25	165.8	0.906	84%
Ie	2- NO_2	$C_{18}H_{10}N_4O_5S$	394.36	123.4	0.40	83%
If	3- NO_2	$C_{18}H_{10}N_4O_5S$	394.36	100.6	0.6190	78%
Ig	4- NO_2	$C_{18}H_{10}N_4O_5S$	394.36	216.7	0.5714	86%
Ih	3,5- NO_2	$C_{18}H_9N_5O_7S$	439.35	168.5	0.7431	85%

Recrystallisation solvent : Methanol

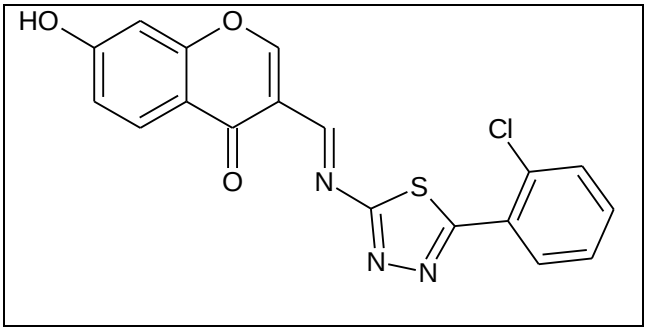
Solvent system for TLC : Chloroform:DMSO(9:1)

Visualizing agent : Iodine vapour

SPECTRAL CHARACTERIZATION DATA[64-66]

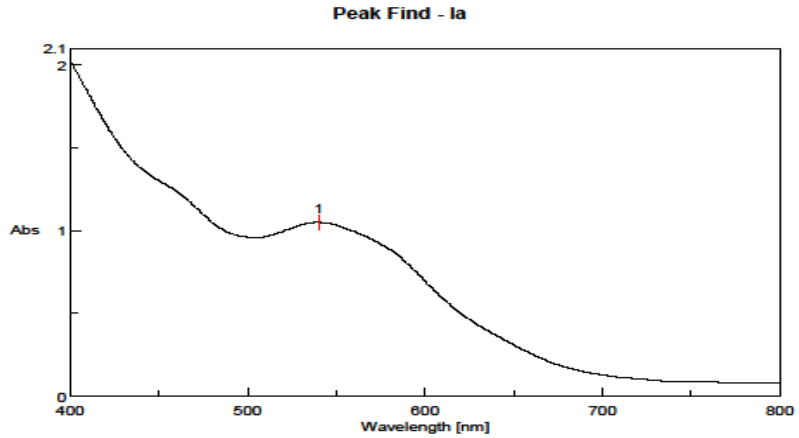
The structures of synthesized compounds during the present investigation were established on the basis of chemical data, IR,UV, NMR and Mass spectral data. The purity of compounds were established by single spot on TLC plates.

Compound code: Ia



Chemical name	3-[(*E*)-{[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 540 nm
IR (KBr,v_{max} in cm^{-1})	3434.6 (Aromatic O-H) 1633.41 (C=O) 1506.13(C=N) 1132.97 (cyclic ester C-O-C) 743.42 (C-S) 1021.12(N-N)

UV SPECTRUM

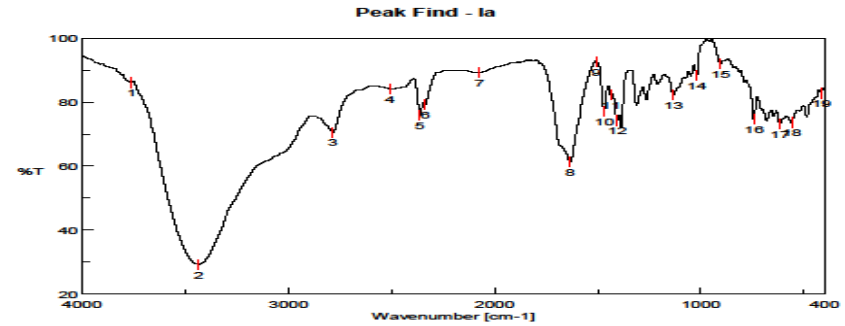


[Comments]
Sample name Ia
Comment uv spectrum
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	540	1.04881			

IR SPECTRUM

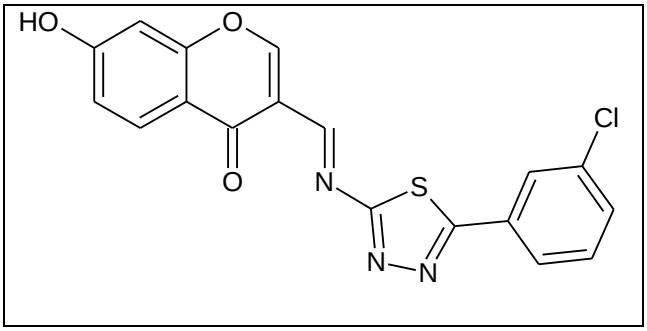


[Comments]
Sample name
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3760.51	86.0248	2	3434.6	29.1375
3	2783.74	70.5327	4	2506.04	83.9951
5	2361.41	75.795	6	2337.3	79.1665
7	2074.07	89.1352	8	1633.41	61.2488
9	1506.13	92.6235	10	1468.53	77.0569
11	1436.71	82.1366	12	1405.85	74.2013
13	1132.97	82.15	14	1021.12	88.2873
15	905.415	92.0022	16	743.424	74.6055
17	617.109	73.1378	18	559.255	73.5216
19	415.585	82.6817			

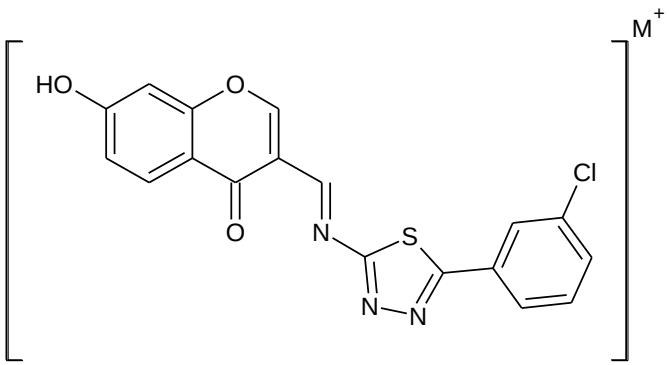
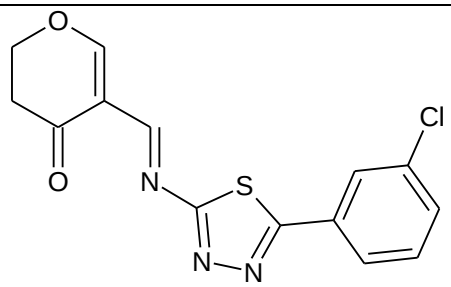
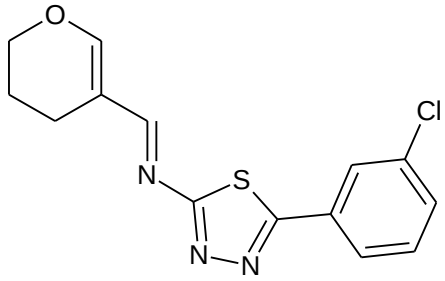
Compound code : Ib



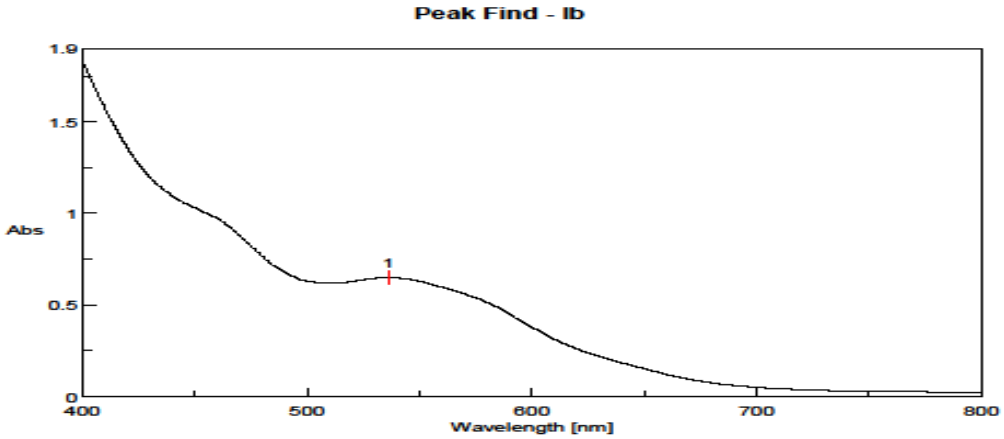
Chemical name	3-[(*E*)-{[5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 536 nm
IR (KBr, v_{max} in cm^{-1})	3428.81 (Aromatic O-H) 1633.41 (C=O) 1533.13(C=N) 1139.72 (cyclic ester C-O-C) 666.285 (C-S- C linkage) 1021.12(N-N)
^{1}H NMR spectral data	13.226 (s, 1H, Ar- OH) 7.526- 7.905 (m, 7H, Ar- H & 1H, CH=C)

Mass Spectral Data

Molecular weight of compoundIb : 383

Sl NO	Fragment	m/z value
1.		383
2.		319
3.		305

UV SPECTRUM



[Comments]
Sample name Ib
Comment uv spectrum
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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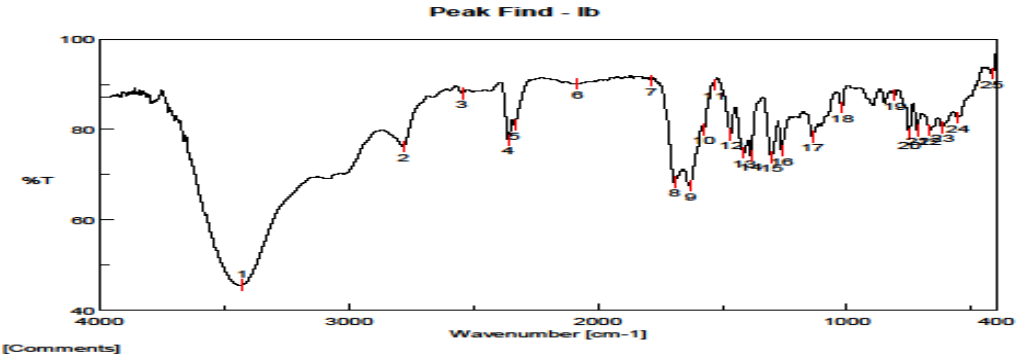
IR SPECTRUM



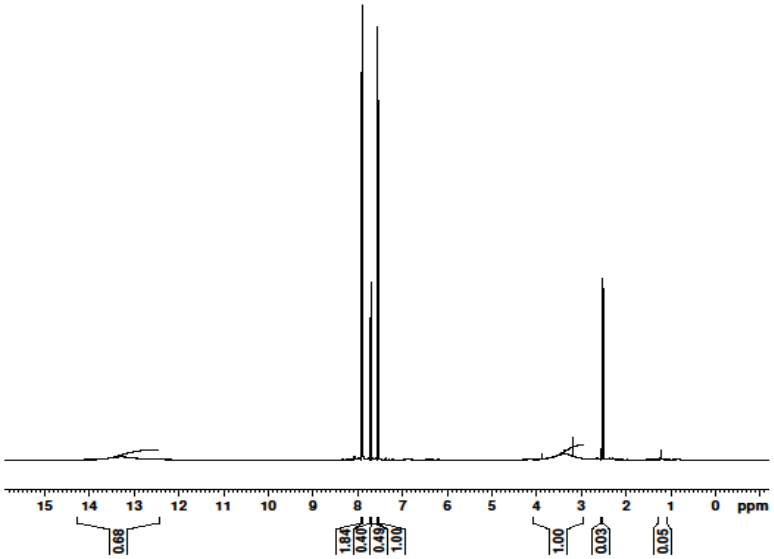
[Comments]
Sample name
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3428.81	45.5611	2	2782.78	76.2567
3	2546.54	87.948	4	2359.48	77.6425
5	2335.37	81.0334	6	2081.78	90.115
7	1783.83	90.8433	8	1692.23	68.338
9	1629.55	67.5969	10	1570.52	79.9656
11	1533.13	89.8355	12	1466.6	78.8337
13	1412.6	74.8703	14	1386.57	74.2156
15	1303.64	73.8302	16	1261.22	75.2485
17	1139.72	78.3655	18	1021.12	84.9018
19	808.992	87.6324	20	750.174	78.9219
21	717.39	79.7573	22	666.285	79.8668
23	617.109	80.4632	24	556.363	82.5635
25	418.477	92.3473			

NMR SPECTRUM

Ib....Geena Mathai



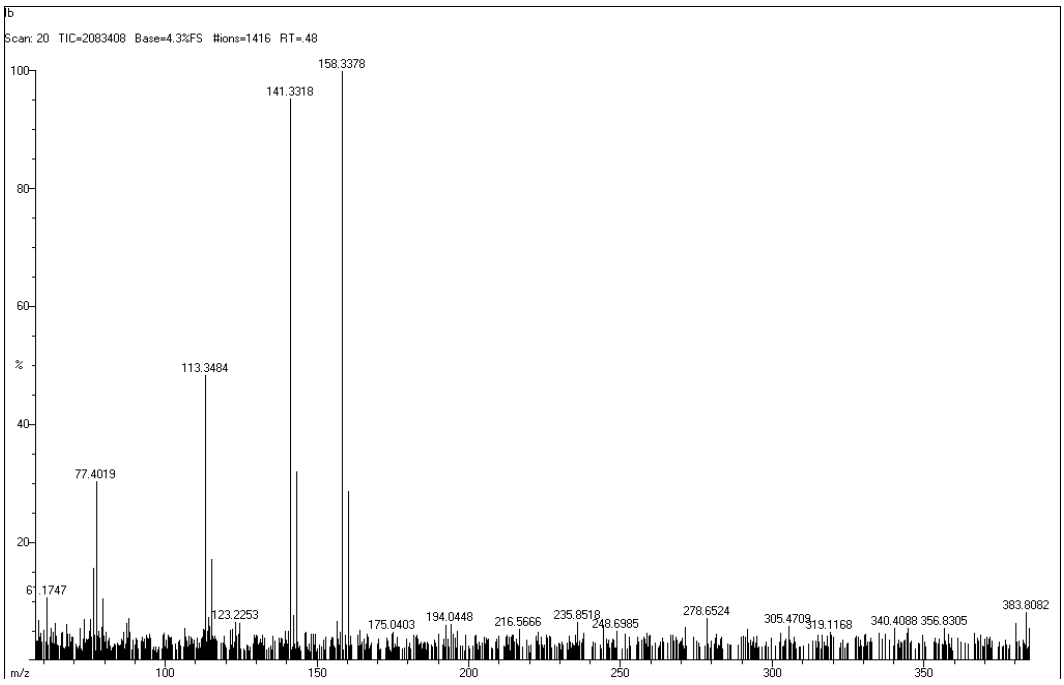
Current Data Parameters
NAME May31-2016
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160531
Time 2.58
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 298.8 K
D1 1.00000000 sec
TD0 1

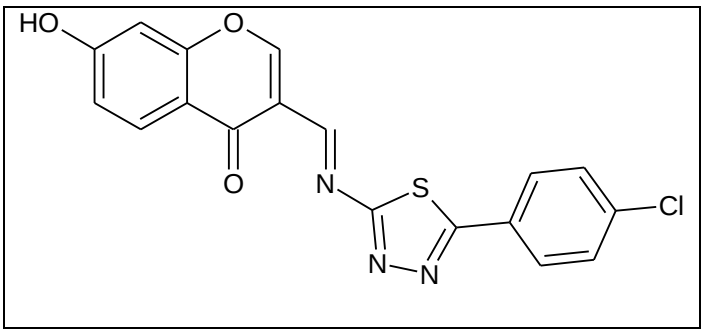
====== CHANNEL f1 ======
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

MASS SPECTRUM



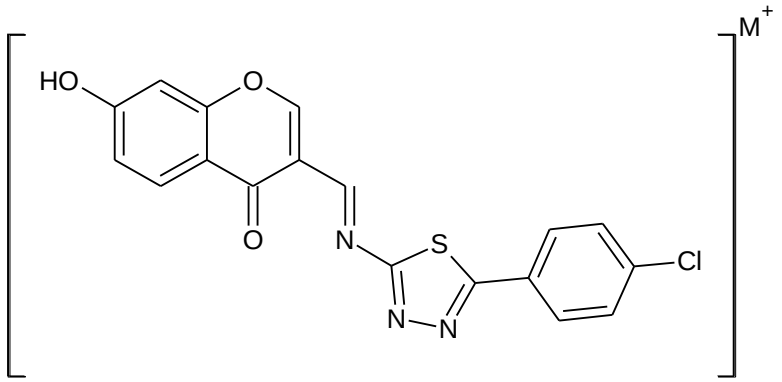
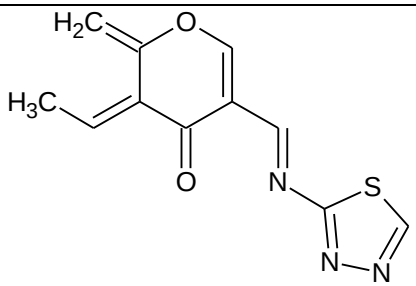
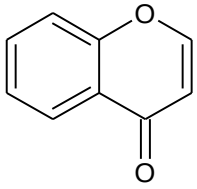
Compound code : Ic



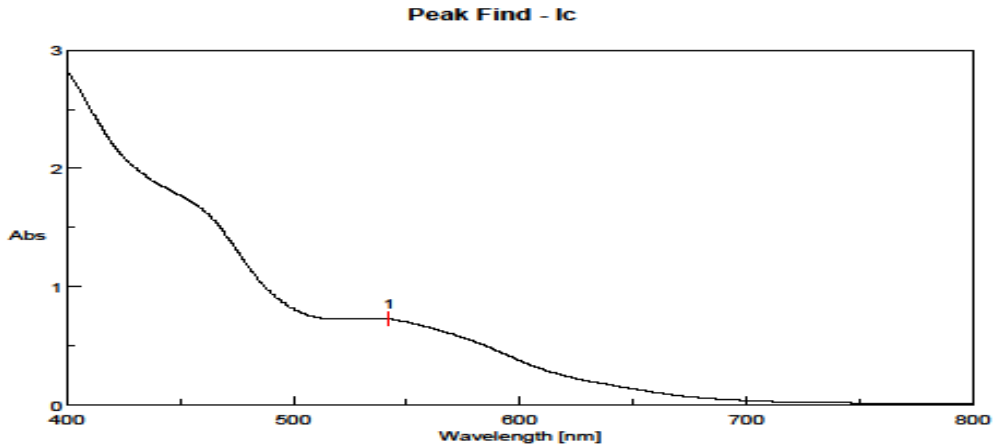
Chemical name	3-[(*E*)-{[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 542 nm
IR (KBr, v_{max} in cm^{-1})	3431.71 (Aromatic O-H) 1629.55 (C=O) 1585.2(C=N) 1107.9 (cyclic ester C-O-C) 768.49 (C-S) 1021.12(N-N)
^{1}H NMR spectral data	7.511-7.999 (m, 6H,Ar-H & 1 H, CH=C) 8.784 (s, 1H,CH=N) 10.115 (s, 1H,Ar –OH)

Mass Spectral Data

Molecular weight of compound Ic : 383

Sl No.	Fragments	m/z
1.		383
2.		247
3		146

UV SPECTRUM



[Comments]
Sample name Ic
Comment uv spectrum
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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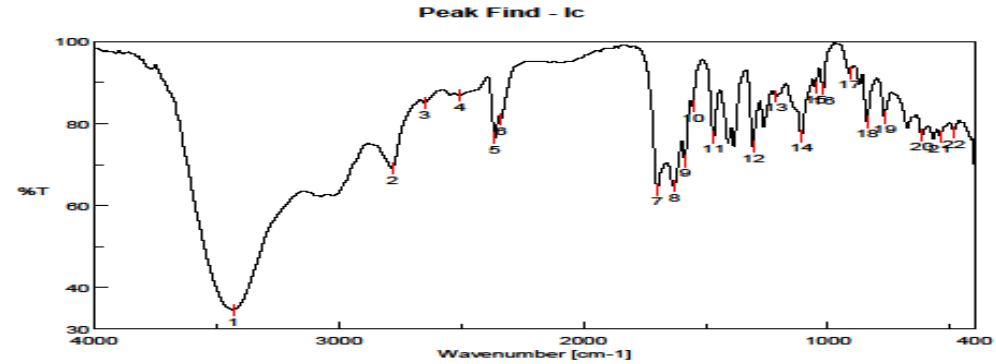
IR SPECTRUM

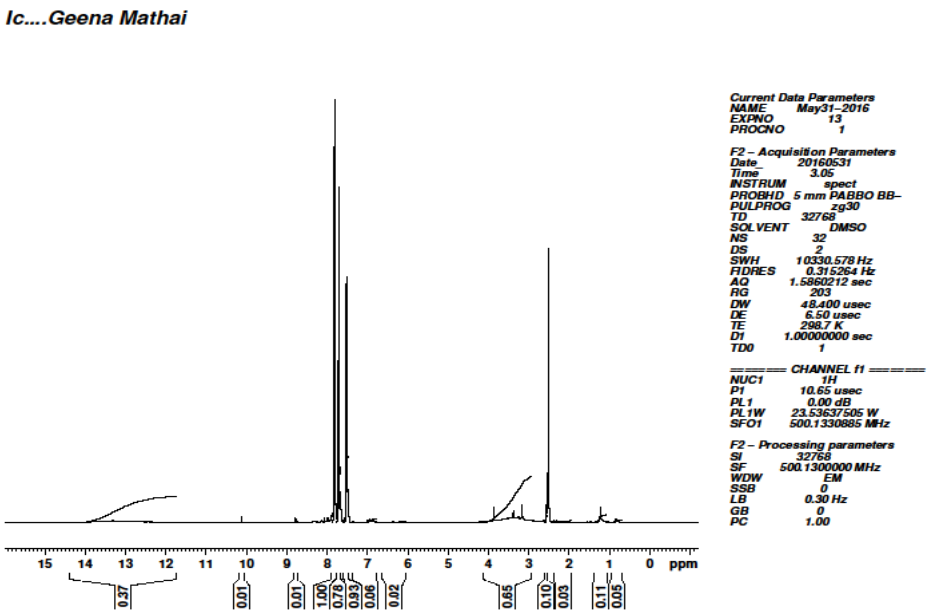


[Comments]
Sample name
Comment
User
Division
Company SRIPMS

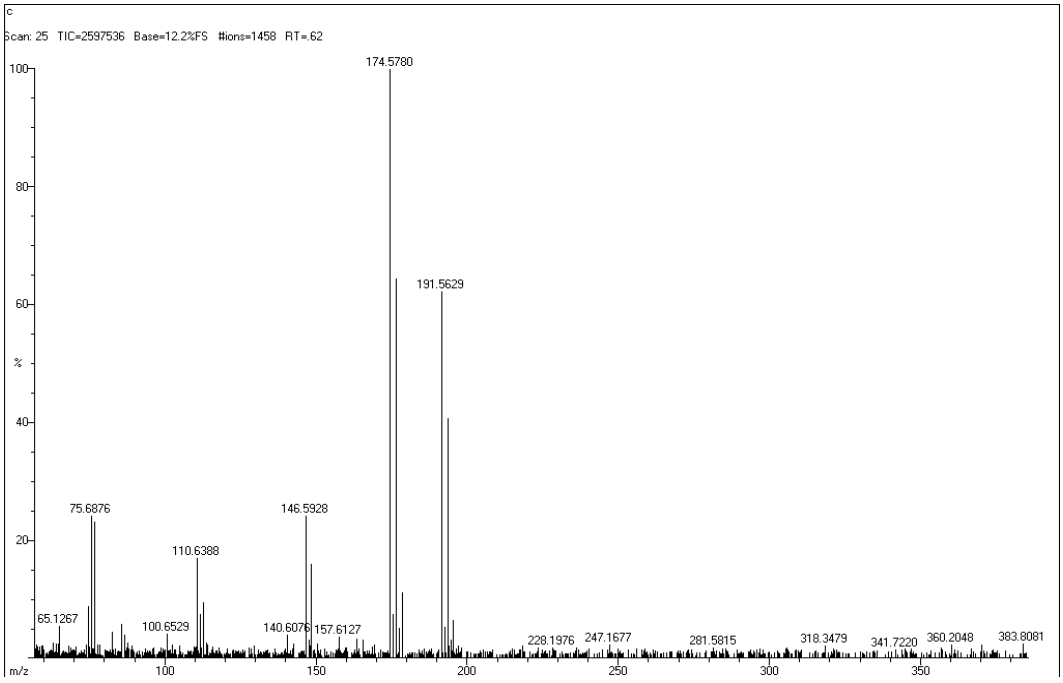
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3431.71	34.5303	2	2782.78	68.999
3	2648.75	85.0435	4	2503.15	86.8666
5	2361.41	76.515	6	2337.3	81.0412
7	1698.02	63.8355	8	1629.55	64.7676
9	1585.2	70.8357	10	1553.38	84.1692
11	1467.56	76.6473	12	1306.54	74.4129
13	1213.01	86.6665	14	1107.9	76.9379
15	1051.01	88.8158	16	1021.12	88.4869
17	912.165	92.2914	18	840.812	80.373
19	768.494	81.383	20	616.145	77.2051
21	541.899	76.8112	22	486.938	77.8818

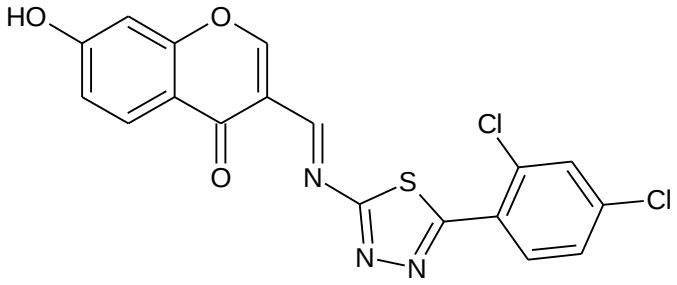
^{1}H NMR SPECTRUM



MASS SPECTRUM

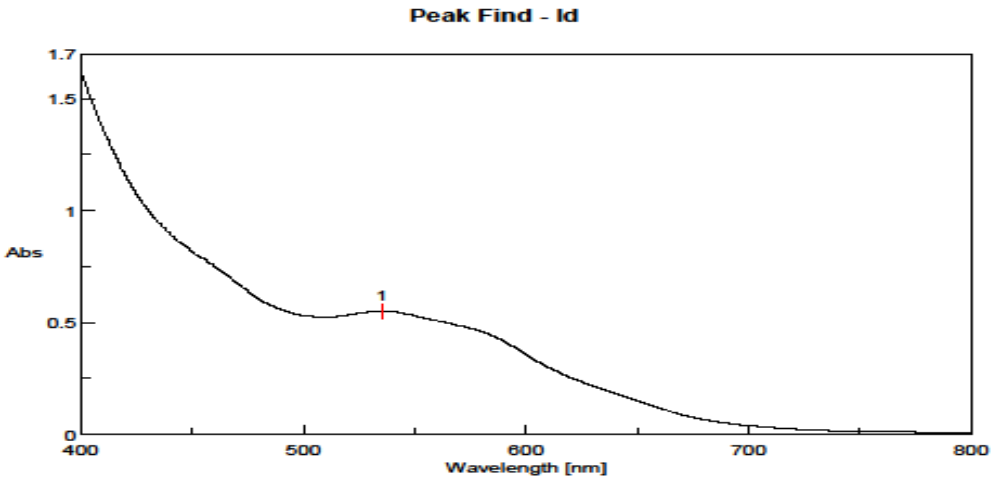


Compound code: Id



Chemical name	3-[(*E*)-{[5-(2,4 -dichlorophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 535 nm
IR (KBr, v_{max} in cm^{-1})	3436.53 (Aromatic O-H) 1634.38 (C=O) 1531.2(C=N) 1127.19 (cyclic ester C-O-C) 762.70 (C-S) 1019.19(N-N)

UV SPECTRUM

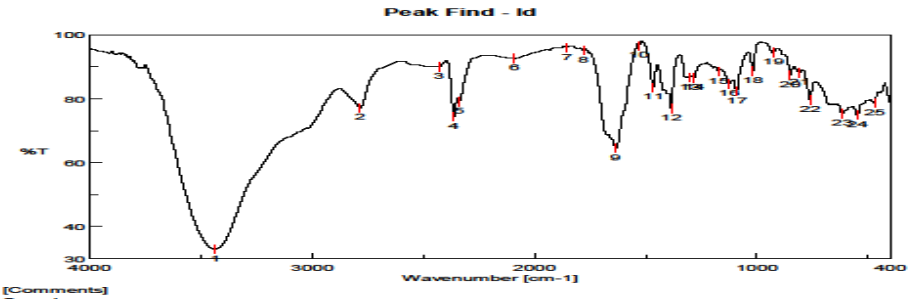


[Comments]
Sample name Id
Comment uv spectrum
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	535	0.549867			

IR SPECTRUM

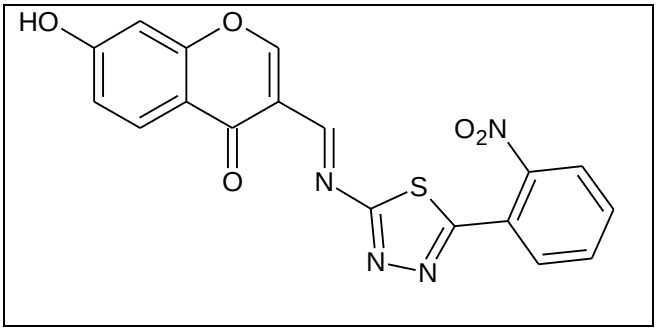


[Comments]
Sample name
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

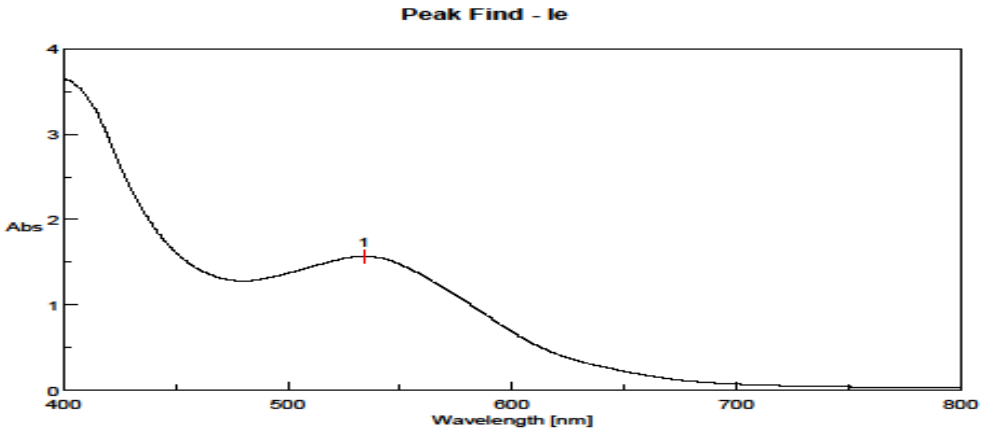
No.	Position	Intensity	No.	Position	Intensity
1	3436.53	32.8422	2	2783.74	77.1171
3	2430.83	89.9591	4	2361.41	74.4134
5	2338.27	79.0612	6	2090.46	92.5318
7	1855.19	95.901	8	1780.94	95.1974
9	1634.38	84.5106	10	1531.2	96.5233
11	1465.63	83.4716	12	1385.6	76.9143
13	1301.72	86.6448	14	1282.43	86.52
15	1172.51	88.4881	16	1127.19	84.5881
17	1091.51	82.3029	18	1019.19	88.6282
19	927.593	94.3772	20	850.454	87.3089
21	809.956	88.1142	22	762.709	79.566
23	616.145	75.3568	24	549.613	74.8099

Compound code : Ie



Chemical name	3-[(*E*)-{[5-(2- nitrophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 534 nm
IR (KBr,v_{max} in cm^{-1})	3432.67 (Aromatic O-H) 1632.45 (C=O) 1532.17(C=N) 640.25 (C-S-C linkage) 1021.12(N-N)

UV SPECTRUM

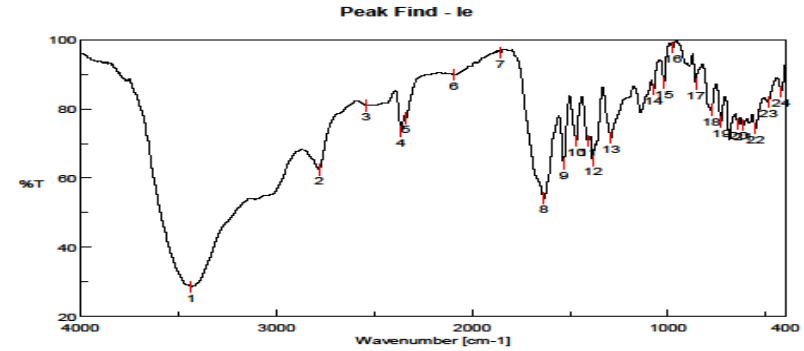


[Comments]
Sample name Ie
Comment uv spectrum
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	534	1.56694			

IR SPECTRUM

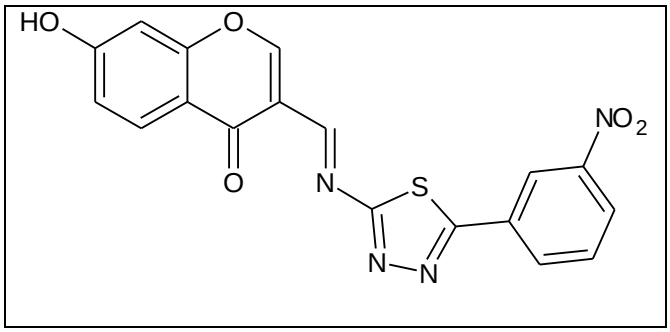


[Comments]
Sample name
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

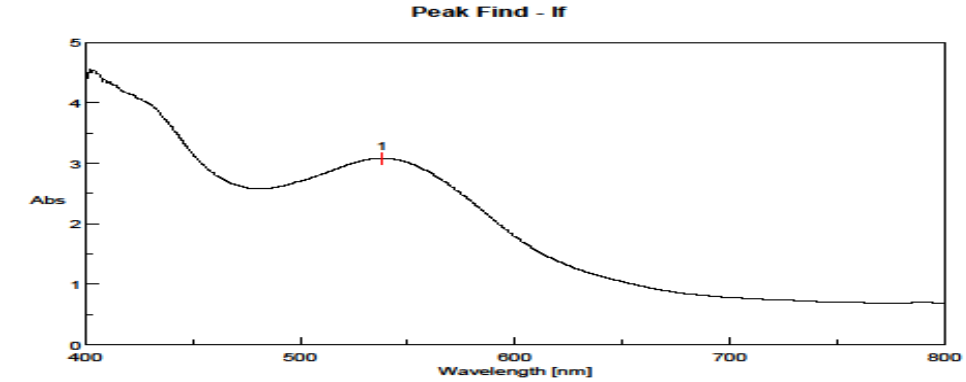
No.	Position	Intensity	No.	Position	Intensity
1	3432.67	28.4972	2	2782.78	62.4384
3	2541.72	80.9225	4	2361.41	73.4101
5	2338.27	77.2089	6	2091.42	89.7906
7	1856.15	96.1608	8	1632.45	54.1385
9	1532.17	64.0638	10	1467.56	70.7176
11	1408.75	70.7226	12	1383.68	65.0309
13	1291.11	71.6069	14	1073.19	85.514
15	1021.12	87.5035	16	976.769	97.7348
17	858.168	87.0988	18	778.136	79.5343
19	728.961	76.1581	20	640.251	75.5869
21	616.145	75.262	22	552.506	74.3531
23	488.866	81.8476	24	422.334	84.9428

Compound code : If



Chemical name	3-[(*E*)-{[5-(3- nitrophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 538 nm
IR (KBr,v_{max} in cm^{-1})	3433.64 (Aromatic O-H) 1626.66 (C=O) 1531.2(C=N) 1134.9 (cyclic ester C-O-C) 652.786 (C-S) 1021.12(N-N)

UV SPECTRUM



[Comments]
Sample name MEROPENEM
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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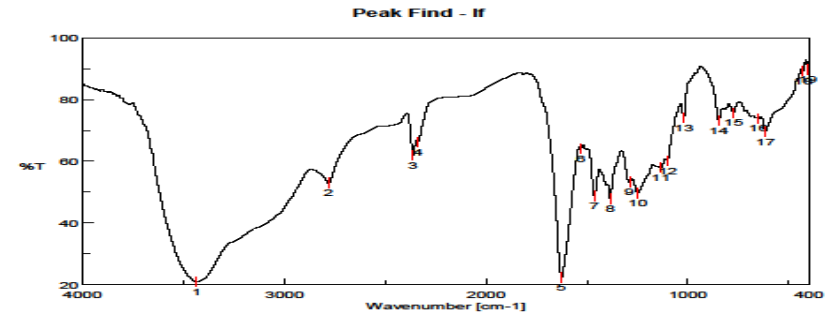
IR SPECTRUM



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User
Division
Company SRIPMS

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7	1463.71	48.6719	8	1384.64	47.7474
9	1290.14	53.2203	10	1249.65	49.644
11	1134.9	58.0147	12	1101.15	60.0885
13	1021.12	74.0518	14	847.561	73.138
15	774.279	75.6701	16	652.786	73.8409
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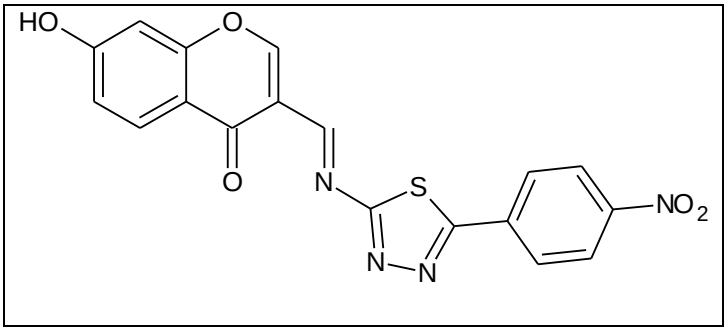
COMPOUND CODE : Ig



Chemical name	3-[(*E*)-{[5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 536 nm
IR (KBr, v_{max} in cm^{-1})	3437.49 (Aromatic O-H) 1629.55 (C=O) 1540.85(C=N) 1106.94 (cyclic ester C-O-C) 715.46 (C-S- C linkage) 1017.27(N-N)
^{1}H NMR spectral data	7.841-8.001 (m, 6H,Ar-H & 1 H, CH=C) 8.784 (s, 1H,CH=N) 10.115 (s, 1H,Ar –OH)

MASS SPECTRAL DATA

Molecular weight of compound Ig : 394

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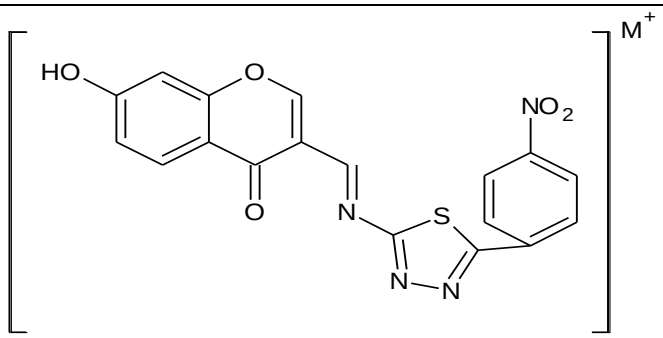
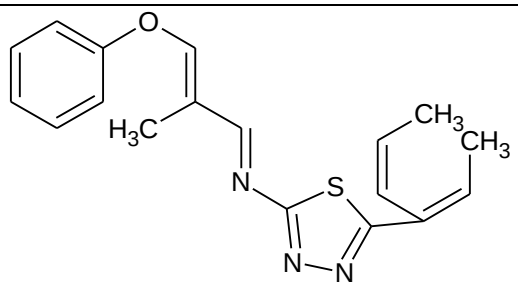
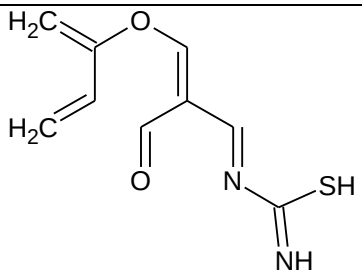
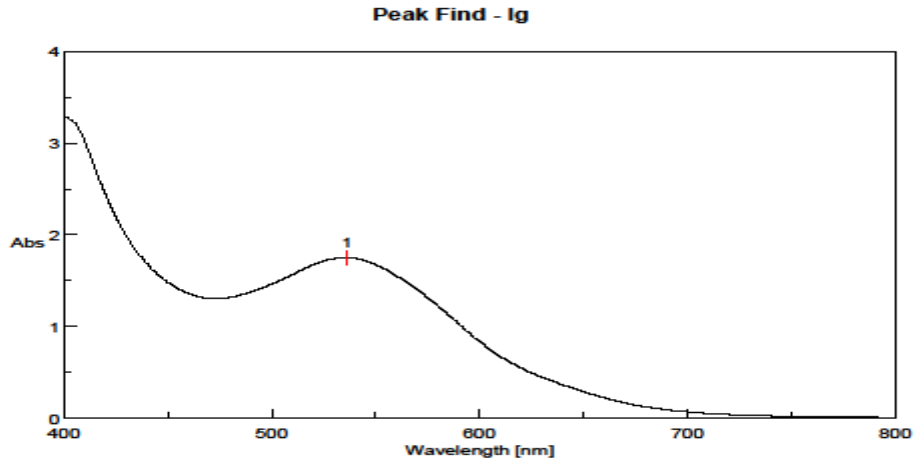
1.		394
2.		325
3.		210

UV spectrum



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Company SRIPMS

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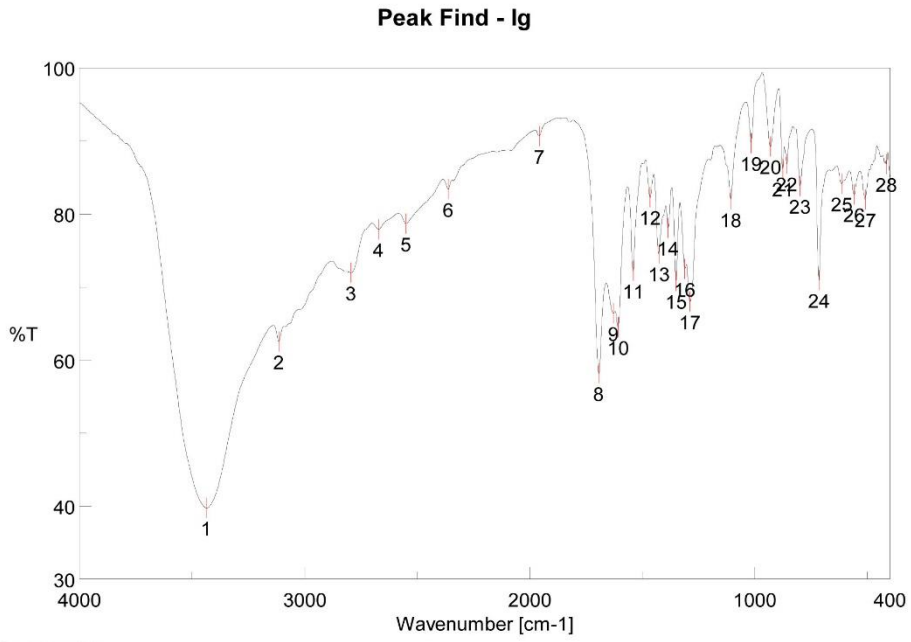
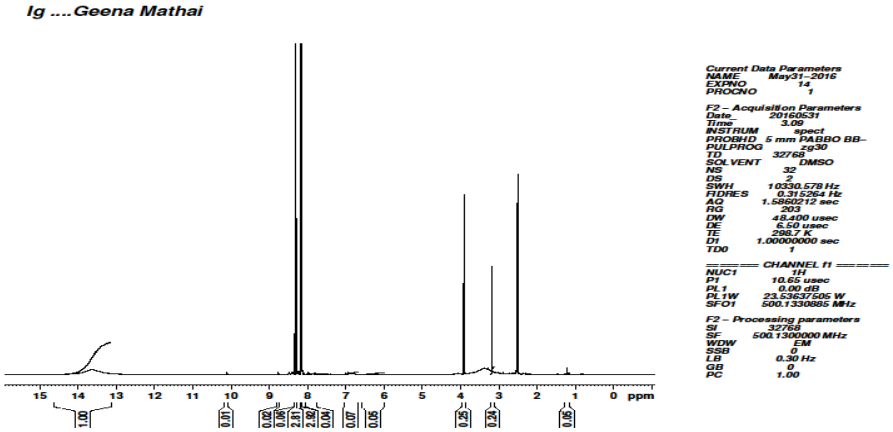


NMR SPECTRUM



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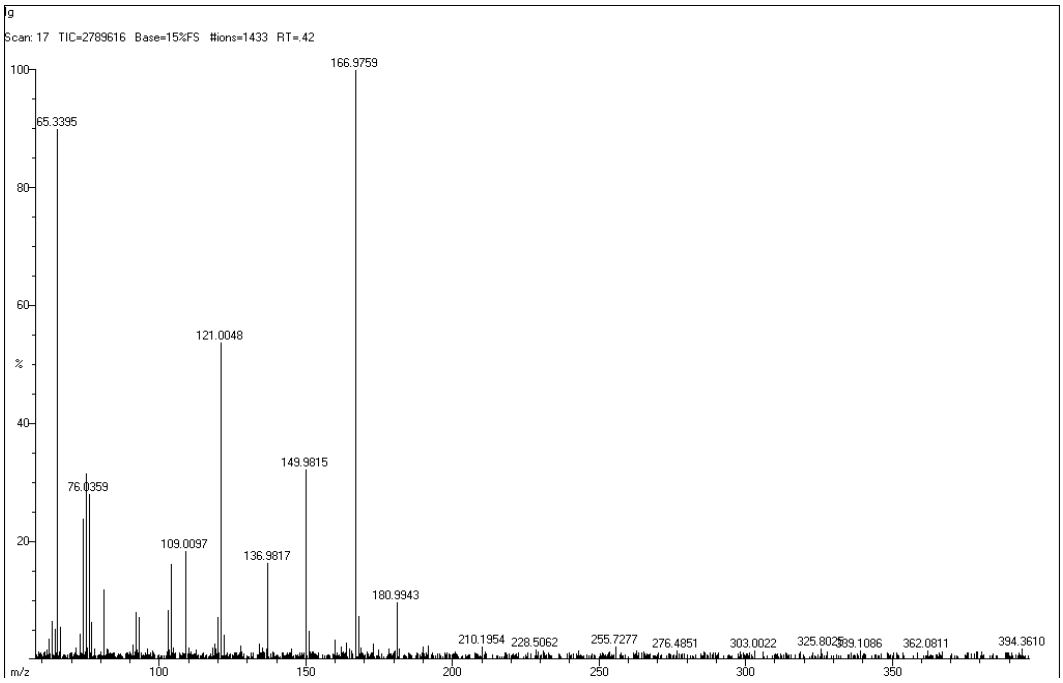
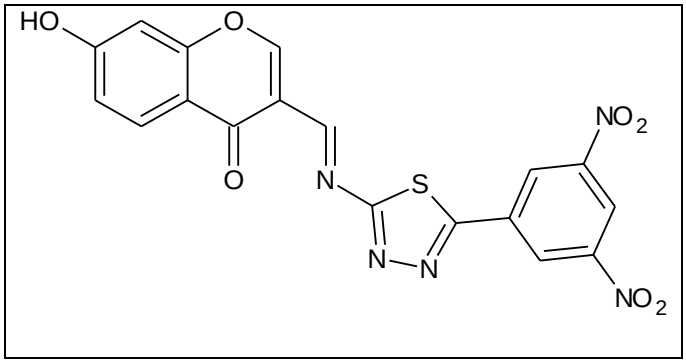


Compound code : Ih



Chemical name	3-[(*E*)-{[5-(2,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl] imino}methyl] -7-hydroxy-4*H*- chromen-4-one
UV spectrum	Solvent used : DMSO λ max : 535 nm
IR (KBr, v_{max} in cm^{-1})	3429.78 (Aromatic O-H) 1628.59 (C=O) 1544.7(C=N) 1096.33 (cyclic ester C-O-C) 695.212 (C-S- C linkage) 1021.12(N-N)
^{1}H NMR spectral data	7.511-7.999 (m, 6H,Ar-H & 1 H, CH=C) 8.784 (s, 1H,CH=N 10.115 (s, 1H,Ar –OH)

Mass Spectral Data

Molecular weight of compound Ih : 439

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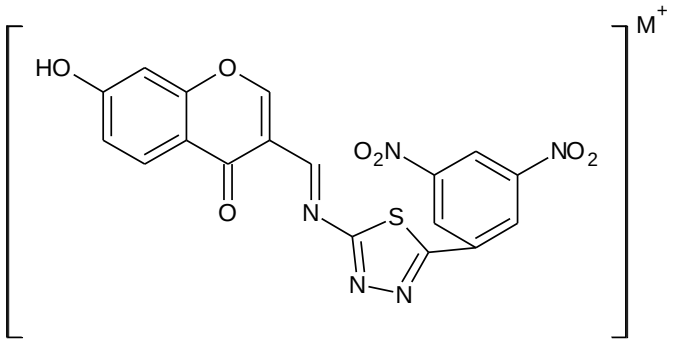
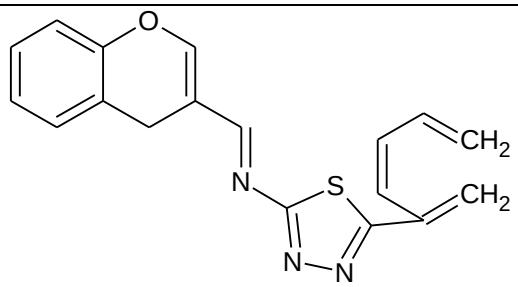
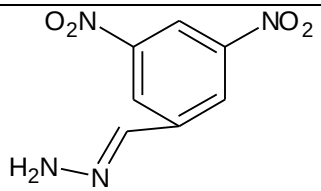
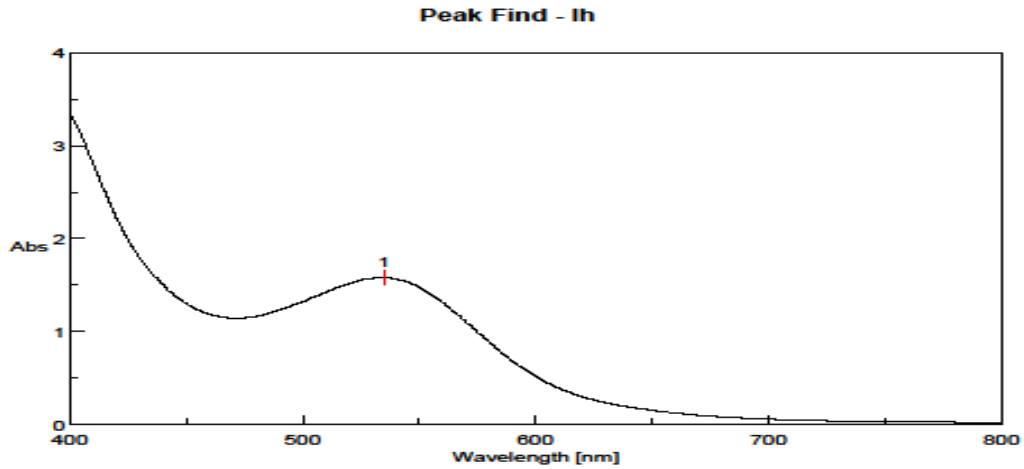
1.		439
2.		321
3.		210

UV SPECTRUM



Comments]
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User
Division
Company SRIPMS

Result of Peak Picking]

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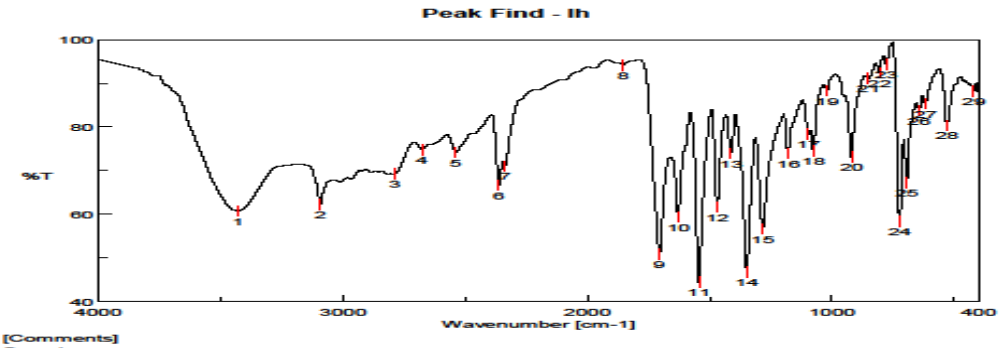
IR SPECTRUM



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Sample name
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User
Division
Company SRIPMS

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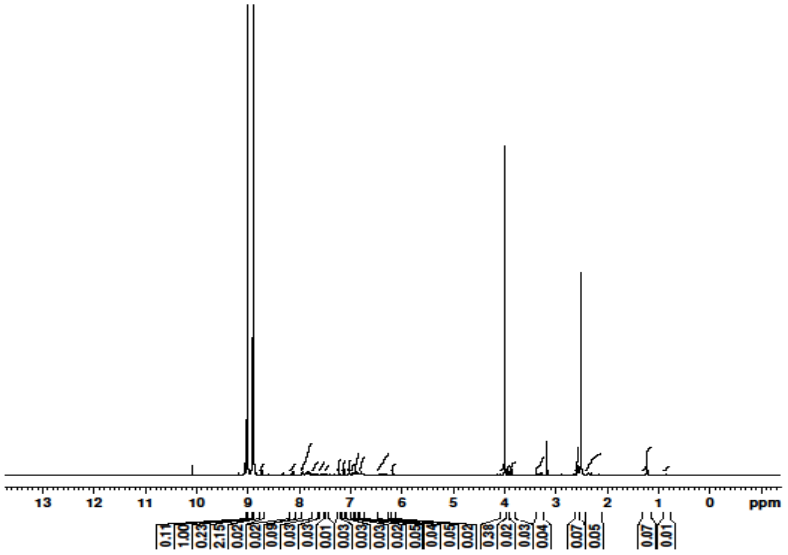
7	2337.3	71.0265	8	1852.29	94.1266
9	1705.73	50.8102	10	1628.59	59.3792
11	1544.7	44.4037	12	1469.49	61.6349
13	1412.6	74.0021	14	1348	46.7266
15	1284.36	56.5883	16	1180.22	73.8859
17	1096.33	78.3549	18	1077.05	74.4876
19	1021.12	88.3185	20	920.843	73.1107
21	851.418	91.1309	22	806.099	92.4367
23	779.101	94.3413	24	724.139	58.3252
25	695.212	67.2126	26	644.108	83.7631
27	618.074	85.3554	28	530.328	80.3892
29	420.406	88.1602			

NMR SPECTRUM

Ih....Geena Mathai

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TE              298.7 K
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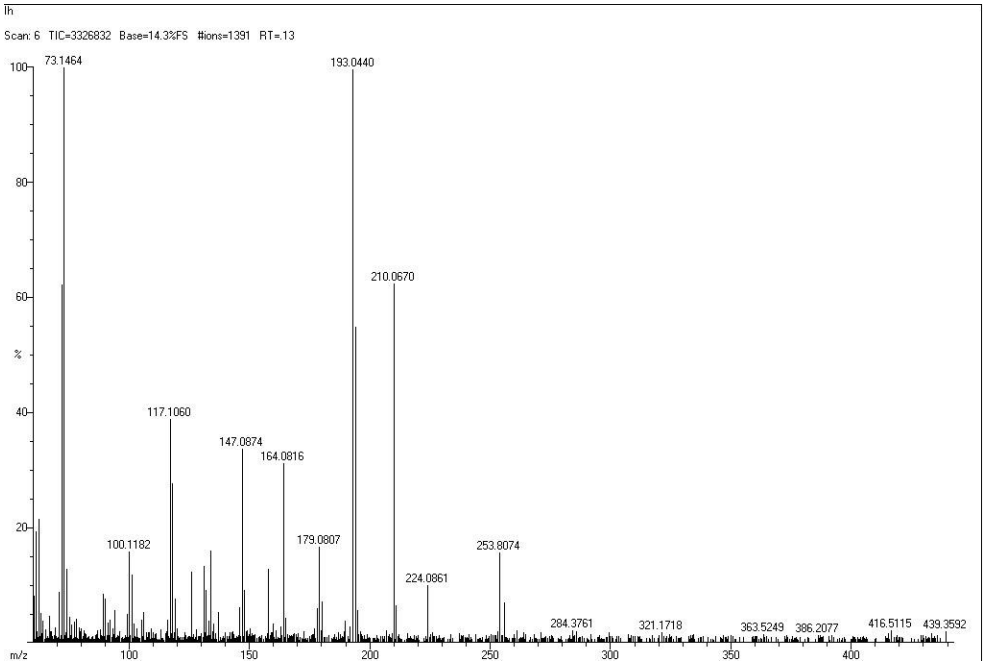


ENZYME INHIBITORY STUDIES[67-69]

***In vitro*Thymidine Phosphorylase Inhibitory Activity**

Principle:

$$\text{Thymidine} + \text{Orthophosphate} \xrightarrow{\text{TP}} \text{Thymine} + \text{2- Deoxy – D-Ribose Phosphate}$$

The rate of decrease in absorbance due to conversion of thymidine to thymine monitored at 290 nm is the rate of enzymatic activity. A comparison of the rate of enzymatic reaction in the absence and presence of inhibitor or test compound would give the relative extent of enzyme inhibition.

Conditions: T = 25^0 C, p^H = 7.4, $A_{290\ nm}$, Light path = 1 cm.

Method : Continuous Spectrophotometric rate determination.

MATERIALS AND METHODS

Chemicals Required

Recombinant *E.coli* thymidine phosphorylase enzyme, potassium phosphate monobasic, anhydrous and Thymidine were obtained from Sigma.

Inhibition of Thymidine phosphorylase by *in vitro* method

REAGENTS:

1. **Reagent A (200 mM Potassium phosphate Buffer, pH 7.4 at 25° C)**

Reagent A was prepared by dissolving 13.61g potassium phosphate, monobasic, anhydrous in deionized water and diluted to 500 ml. 125 ml of above solution was transferred to 500 ml standard flask and added 39.1 ml

1M NaOH to adjust p^H 7.4 at 25^0 C. Made up to final volume with deionized water.

2. Reagent B (5 mM Thymidine solution)

Reagent B was prepared by dissolving 0.1211g Thymidine in 100 ml Reagent A.

3. Reagent C (10 mM potassium phosphate buffer, pH 7 at 25°C-Enzyme Diluent)

Reagent C was prepared by dissolving 1.361g potassium phosphate,monobasic, anhydrous in deionized water and diluted to 1000 ml. 25 ml of above solution was transferred to 100 ml standard flask and added 14.55 ml 1M NaOH to adjust p^H 7.4 at 25° C. Made up to final volume with deionized water.

4. Reagent D (Thymidine phosphorylase enzyme solution)

Immediately before use, prepare a solution containing 1.5 units/ml of Thymidine Phosphorylase in cold Reagent C.

PROCEDURE

The enzymatic assay was performed at room temperature in a JASCO V-530 UV/VIS spectrophotometer using a detection wavelength of 290 nm. 10 µl of TP solution (1.5 U/ml in p^H 7.0 phosphate buffer),10 µl of the test compound solution in DMSO (for blank, 10 µl DMSO was added) and 200 µl of thymidine solution (5mM in p^H 7.4 phosphate buffer) were added to 780 µl of phosphate buffer (p^H 7.4) in a 1.5 ml cuvette. After gentle shaking by inversion of the cuvette, the absorbance values at 4 min, 8 min, 12 min, 16 min and 20 min were recorded successively. These absorbance values were plotted against time and linear regression was performed to obtain the slope of the line plotted which was taken to be the

velocity of enzyme catalyzed reaction. The percentage inhibition at this concentration was calculated by dividing the difference between the enzymatic velocity of the blank and the enzymatic velocity in the presence of the inhibitor (or test compound) by the enzymatic velocity of the blank. To determine the IC_{50} value of a certain compound, percentage inhibitions of at least 7 different concentrations that span over the estimated IC_{50} values were determined by the method mentioned above and plotted against concentration using Graph Pad Instat software. The IC_{50} value was determined as the concentration of the inhibitor that caused 50% inhibition. Assay was done in triplicate and the results were expressed as standard error of mean.

$$\% \text{ inhibition} = \frac{A_0 - A_1}{A_0}$$

A_0= Enzymatic velocity of blank

A_1= Enzymatic velocity of test compounds

DEFINITION OF UNIT

One unit of TP will convert 1 µmole each of thymidine and phosphate to thymine and 2 α deoxy ribose 1 phosphate per minute at p^H 7.4 at 25˚C.

Table 2 :*In vitro* thymidine phosphorylase inhibitory activity of compounds Ia -Ih

Compound code	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml	320 µg/ml	640 µg/ml	IC_{50} µM
Ia	39.91±0.1933	42.63±0.1933	48.63±0.1933	60.07±0.1933	69.98±0.1933	74.80±0.1967	78.86±0.1933	**63.63±3.6**
Ib	38.75± 0.1933	43.79±0.1933	49.41±0.3378	56.03±0.1933	62.00±0.1933	68.98±0.1933	75.19±0.1933	**154.97±4.1**
Ic	41.39±0.0633	43.6±0.5800	50.18±0.0933	59.10±0.3867	71.11±0.0933	82.26±0.1933	88.56±0.1933	**29.13±2.1**
Id	40.30±0.1933	43.21±0.1933	49.60±0.1967	61.42±0.1933	68.21±0.1933	74.21±0.1933	79.45±0.1967	**39.13±3.5**
Ie	38.17±0.3867	44.37±0.1933	49.02±0.1933	62.59±0.1967	69.63±0.2267	72.67±0.3349	78.86±0.1933	**41.08±3.2**
If	38.75±0.1933	42.05±0.1933	47.47±0.1933	61.42±0.1933	68.40±0.1933	74.80±0.1967	76.54±0.1933	**74.18±2.72**
Ig	40.81±0.5233	43.21±0.1933	49.99±0.3378	60.07±0.3867	75.77±0.1933	85.85±0.1967	93.35±0.1233	**23.36±1.16**
Ih	35.84±0.1933	43.98±0.1933	48.25±0.3349	57.93±0.1933	67.63±0.1933	71.70±0.1933	74.02±0.1933	**106.7±3.49**
7-Deazaxanthine (Standard)								**39.28±0.76**[3]

All the determinations were carried out in triplicate and the values are expressed as the mean± SEM

INFERENCE:

Invitro thymidine phosphorylase inhibitory activity was determined for the synthesized compounds, Ia-Ih at concentrations ranging from 10-640 µg/ml. A dose dependent increase in percentage of inhibition to thymidine phosphorylase was observed for all the synthesized compounds.

Compounds Ia-Ih exhibited varying degrees of thymidine phosphorylase inhibitory activity with IC_{50} values in the range of 23- 154 µM when compared with standard drug 7- deazaxanthine having an IC_{50} value of 39.28±0.76 µM.

Compound Ig(IC_{50} = 23.36±1.160) and Ic (29.13±2.1) were found to be the most active members of the series , more potent than the standard.

Compound Id (IC_{50} =39.13± 3.5) and Ie (41.08± 3.2) also showed excellent anti- thymidine phosphorylase activity comparable with the standard.

Compounds Ib and Ih showed IC_{50} values greater than 100 µM, therefore considered to be inactive.

Activity of compound largely depends upon the substitution on aromatic ring attatched at the 2 nd position of 1,3,4 thiadiazole.Compound Ig with para nitro substitution on phenyl ring showed the highest degree of thymidine phosphorylase inhibitory activity .

Compound Ic with para chloro substitution on phenyl ring and compound Id with with ortho and para dichloro substitutions on phenyl moiety showed IC_{50} value less than the standard.

The substitutions on position 4 of the phenyl ring appeared to be an important contributory factor to the inhibitory activity.

Compound Ig was more potent than Ic suggested that greater electron withdrawing effect would lead to more active compounds. Therefore, the more electron withdrawing substituents inserted on para position of phenyl ring will result in more potent compounds.

Substitution of chloro with nitro in the ortho, meta and para positions of phenyl ring resulted in increase in inhibitory activity. (Ie> Ia, If> Ib, Ig> Ic).

Introduction of two nitro groups in the phenyl ring resulted in loss of activity.

Introduction of chloro group in meta position also resulted in loss of activity.

The order of potency of compounds was found to be Ig> Ic> Id> Ie> Ia> If> Ih> Ib.

ANTIMICROBIAL SCREENING

APPARATUS AND CHEMICALS REQUIRED

Hot air oven	:	Technico
Incubator	:	Technico
Autoclave	:	Kailash
Horizontal laminar flow hood	:	CLEAN AIR instruments Inc.
Petri dishes	:	SD Fine Chem Ltd
Physical balance	:	Hrory (pvt) limited
Micropipettes	:	VARI pipettes (Hi- Tab Lab)
Microtips	:	Tarsons
Conical flask(250 ml)	:	Borosil
Test tubes	:	Borosil
Sterile swab	:	Hi media
Nutrient broth	:	Hi Media
Agar powder	:	Hi Media

The antibacterial screening were carried out in the Pharmaceutical Biotechnology Laboratory, College Of Pharmacy, SRIPMS, Coimbatore.

SCREENING FOR ANTIBACTERIAL ACTIVITY[70,71]

Media : Mueller- Hinton Agar

Muller Hinton broth gelled by the addition of 2% agar (bacteriological grade)

Ingredients

Casein enzymatic hydrosylate	:	17.5 gm/L
Beef infusion	:	300 gm/L
Soluble starch	:	1.5 gm/L
Final pH at 25° C	:	7.4 $\pm$0.2

Preparation

The ingredients were dissolved in distilled water with the aid of heat and p^H was adjusted to 7.4$\pm$0.2 by using dilute acid or alkali.

Sterilization

15-30 ml of Mueller Hinton agar was transferred to petriplates and sealed. It was then autoclaved at a pressure of 15 psi (121^0C) for not less than 15 minutes.

Microorganisms used

Staphylococcus aureus NCIM 2079, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2918, *Pseudomonas aeruginosa* NCIM 2036 were procured from National Chemical Laboratory, Pune and stored in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy,SRIPMS, Coimbatore. The strains were confirmed for their purity and identified by Gram's staining method and their characteristic biochemical reactions. The selected strains were preserved by sub culturing periodically on nutrient agar slants and storing them under frozen conditions. For antimicrobial study fresh 24 hr cultures were used after the standardization of culture.

Working conditions

The entire work was done by using horizontal laminar flow hood so as to provide aseptic conditions. Before the commencement of work air sampling was carried out using a sterile nutrient agar plate and exposing it to the environment inside the hood. After incubation it was checked for the growth of microorganism and absence of growth confirmed aseptic working condition.

Preparation of Inoculam

The inoculams for the experiment was prepared fresh in Mueller Hinton broth from preserved frozen slants. It was incubated at 37° C for 18- 24 hrs and used after standardization.

Standardization of Inoculums

All the organisms were grown overnight (24 hours) at 37^0 C on nutrient agar and harvested during the stationary phase. Active cultures for experiments were prepared by transforming a loop full of cells from the stock culture to the test tubes containing Muller Hinton broth, incubated for 24 hrs at 37^0C. Inoculum was standardized by matching the turbidity of the culture to 0.5 McFarland standard. The standard was produced by mixing 0.5 ml of 0.048 $BaCl_2$(1.175%w/v Barium chloride dehydrates) with 99.5 ml of 0.36N H_2SO_4. If the turbidity of culture matches that of McFarland standard, the culture inoculating suspension has approximately $2x10^6$ CFU/ml of bacteria.

Drugs used	:	Synthesized drugs
Standard drugs	:	Ciprofloxacin(5µg)
Solvent	:	Dimethyl sulfoxide

ANTI- BACTERIA SCREENING BY KIRBY-BAUER METHOD[72]

Mueller Hinton agar was prepared aseptically to get a thickness of 5-6 mm.The plates were allowed to solidify and inverted to prevent the condensate falling on the agar plate surface. The plates were dried at 37^0Cbefore inoculation.The organisms were inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculums and excess of inoculums was removed by pressing and rotating the swab firmly against the sides of the culture tube above the level of liquid and finally streaking all over the surface of the medium three times, rotating the plates through an angle of 60° after each application. Finally the swab was pressed round the edges of agar surface. The plates was allowed to dry at room temperature, with the lid closed. The measured quantity of test drugs, standard and blank was poured in the wells, where the wells are made with the help of borer. The plates were kept in the refrigerator for 1 hour to facilitate the diffusion of the drugs. Plates were prepared in triplicate and they were incubated for 18-24 hours at 37˚C. After the incubation, the diameter of the zone of inhibition for each drug was measured and compared with that of standard. All the synthesized compounds were tested for antibacterial activity against Gram positive and Gram negative bacteria. Saturated compounds of the solutions were first studied and then the compounds with zone of inhibition greater than 15 mm were taken for quantitative studies.

Table :3 QUANTITATIVE SCREENING OF TEST COMPOUNDS FOR ANTIBACTERIAL ACTIVITY AGAINST GRAM POSITIVE ORGANISMS

Sl.No	Compound code	Diameter of zone of inhibition in mm			
		***Staphylococcus aureus* NCIM 2079**		***Bacillus subtilis* NCIM 2063**	
		500 µg/ ml	250 µg/ ml	500 µg/ ml	250 µg/ ml
1	Ia	14	13	12	11
2	Ib	15	14	15	14
3	Ic	15	14	13	12
4	Id	15	14	13	12
5	Ie	14	12	12	11
6	If	15	13	13	12
7	Ig	15	13	14	12
8	Ih	16	15	13	11
9	Blank(DMF)	-	-	-	-
10	Standard Ciprofloxacin (5µg/ml)	34	34	26	26

Table :4 QUANTITATIVE SCREENING OF TEST COMPOUNDS FOR ANTIBACTERIAL ACTIVITY AGAINST GRAM NEGATIVE ORGANISMS

Sl.No	Compound code	Diameter of zone of inhibition			
		Escherichia coli NCIM 2918		*Pseudomonas aeruginosa*NCIM 2036	
		500 µg/ ml	250 µg/ ml	500 µg/ ml	250 µg/ ml
1	Ia	15	12	13	12
2	Ib	15	14	14	13
3	Ic	14	13	13	12
4	Id	18	12	15	13
5	Ie	18	14	15	14
6	If	19	15	13	12
7	Ig	19	18	15	14
8	Ih	14	13	18	16
9	Blank(DMF)	-	-	-	-
10	Standard Ciprofloxacin (5µg/ml)	32	32	33	33

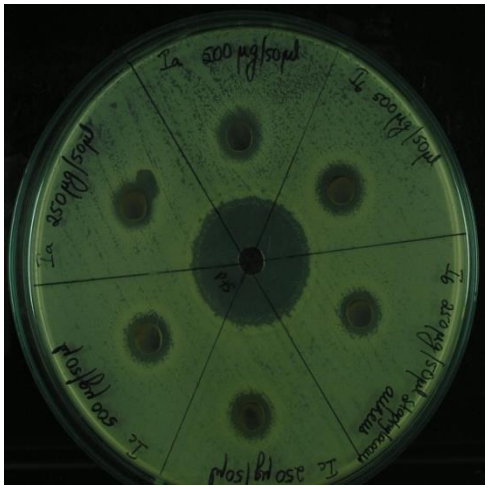
SCREENING OF SYNTHESIZED COMPOUNDS FOR ACTIVITY AGAINST GRAM POSITIVE ORGANISM

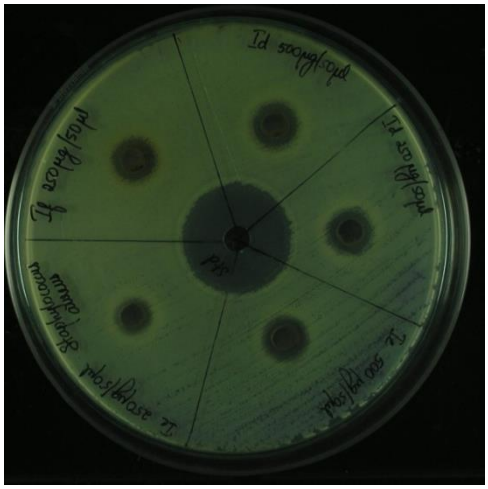
Concentrations used : 500 µg/ml and 250 µg/ml.

Standard drug : Ciprofloxacin (5 µg/ml)

Solvent used : Dimethyl formamide

Zone of inhibition of compounds against *Staphylococcus aureus* NCIM 2079





Zone of inhibition of compounds against *Bacillus subtilis* NCIM 2063

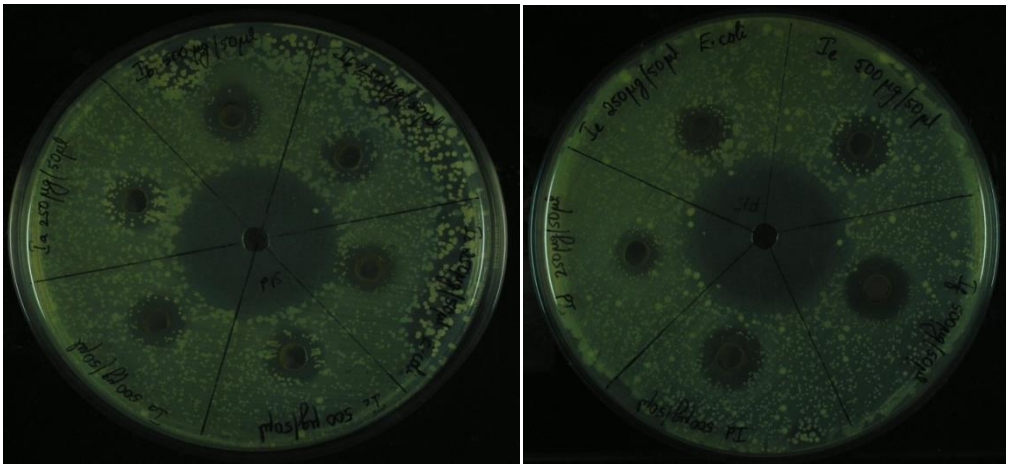
SCREENING OF SYNTHESIZED COMPOUNDS FOR ACTIVITY AGAINST GRAM NEGATIVE ORGANISM

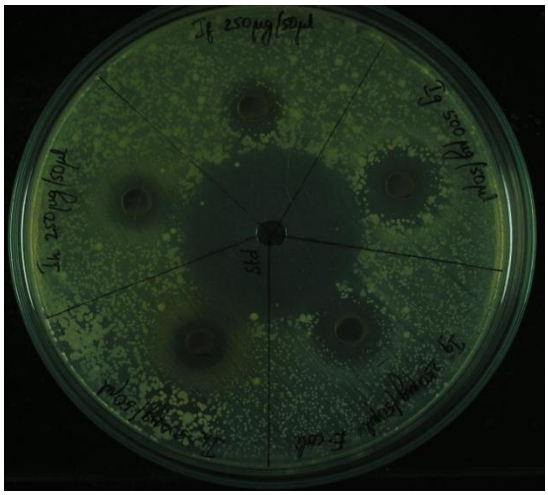
Concentrations used : 500 µg/ml.

Standard drug : 5 µg/ml.

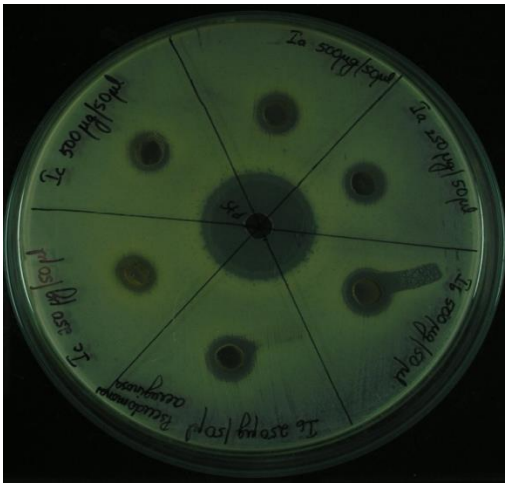
Solvent used : Dimethyl formamide

Zone of inhibition of compounds against *Escherichia coli* NCIM 2918





Zone of inhibition of compounds against *Pseudomonas aeruginosa* NCIM 2036



DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION[73]

Compounds with good activity were selected for the determination

of minimum inhibitory concentration.

Preparation of inoculums

The inoculums for the experiment was prepared fresh in Mueller Hinton broth from the preserved frozen slant culture. It was incubated for 18-24 hrs and used for the study after dilution to give 1:10 or 1:100 dilutions.

Standardization of inoculums

The suspension of test organisms used was diluted to 1:100 after overnight incubation and standard drop(0.01 ml) was used for MIC determination.

***In vitro* determination of MIC**

Invitro MIC determination of the test samples was carried out by two fold serial dilution technique in Mueller Hinton broth for *Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2918, *Pseudomonas aeruginosa* NCIM 2036 .The concentration range of the compounds used were 5000- 78.15 µg/ml.

Procedure

- Test tubes were numbered as 1-8 and 1 ml of Mueller Hinton broth was added to each tubes. They were autoclaved at a pressure of 15 psi at 121° C for not less than 15 minutes.
- 1 ml of diluted stock solution (5000 µg/ml) was added to the first test tube and serially transfer 1 ml up to the 7^{th} test tube to obtain the quantities indicated.
- From the 7^{th} test tube 1 ml was discarded.
- The 8^{th} test tube was used as the control.
- Diluted broth culture of test organism (0.01 ml) was added to all the

test tubes including the control with a standard micropipette.

- Mixed gently and incubated at 37^0 C for 16-18 hrs.
- Results were observed.
- The above procedure was carried out in duplicate.
- The MIC was interpreted as the highest dilution of the test compound which showed clear fluid with no development of turbidity.

Table:5

Tube No.	1	2	3	4	5	6	7	Control
Mueller Hinton broth(ml)	1	1	1	1	1	1	1	1
Drugs in serial dilution(ml)	1	1	1	1	1	1	1	-
Discard (ml)	-	-	-	-	-	-	1	-
Culture	Add 0.01 ml to each tube , Mix gently, kept in incubator for 16-18 hrs							-
Concentration (µg/ml)	5000	2500	1250	625	312.5	156.25	78.15	-

Table :6 SCREENING OF MINIMAL INHIBITORY CONCENTRATION FOR SAMPLE WITH GRAM POSITIVE BACTERIA (*Staphylococcus aureus* NCIM 2079)

Compound code	Concentration(µg/ml)							
	5000	2500	1250	625	312.5	156.25	78.15	MIC values (µg/ml)
Id	-	-	-	-	-	+	+	312.5
Ie	-	-	-	-	+	+	+	625
If	-	-	-	-	-	+	+	312.5
Ig	-	-	-	-	-	+	+	312.5
Ih	-	-	-	-	+	+	+	625
Control	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	

Table :7 SCREENING OF MINIMAL INHIBITORY CONCENTRATION FOR SAMPLE WITH GRAM POSITIVE BACTERIA (*Bacillus subtilis* NCIM 2063)

Compound code	Concentration(µg/ml)							
	5000	2500	1250	625	312.5	156.25	78.15	MIC values (µg/ml)
Id	-	-	-	-	-	+	+	312.5
Ie	-	-	-	-	-	+	+	1250
If	-	-	-	+	+	+	+	312.5
Ig	-	-	-	-	+	+	+	625
Ih	-	-	-	-	-	-	+	156.25
Control	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	

(+) indicates turbidity

(-) indicates clear

Table :8 SCREENING OF MINIMAL INHIBITORY CONCENTRATION FOR SAMPLE WITH GRAM NEGATIVE BACTERIA (*Escherichia coli* NCIM 2918)

Compound code	Concentration(µg/ml)							
	5000	**2500**	**1250**	**625**	**312.5**	**156.25**	**78.15**	**MIC values (µg/ml)**
Id	-	-	-	-	-	+	+	312.5
Ie	-	-	-	+	+	+	+	1250
If	-	-	-	-	+	+	+	625
Ig	-	-	-	-	-	-	+	156.25
Ih	-	-	-	-	-	+	+	312.5
Control	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	

Table : 9 SCREENING OF MINIMAL INHIBITORY CONCENTRATION FOR SAMPLE WITH GRAM NEGATIVE BACTERIA (*Pseudomonas aeruginosa* NCIM 2036)

Compound code	Concentration(µg/ml)							
	5000	2500	1250	625	312.5	156.25	78.15	MIC values (µg/ml)
Id	-	-	-	-	+	+	+	625
Ie	-	-	-	+	+	+	+	1250
If	-	-	-	-	+	+	+	625
Ig	-	-	-	-	+	+	+	625
Ih	-	-	-	-	-	+	+	312.5
Control	-	-	-	-	-	-	-	
Blank	+	+	+	+	+	+	+	

(+) indicates turbidity

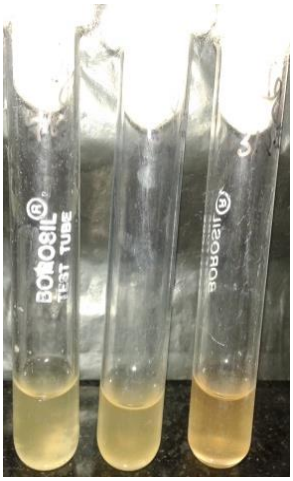
(-) indicates clear

***In vitro* MIC determination against *Staphylococcus aureus* NCIM 2079**

Compound :Id

Compound: Ie

Compound: If

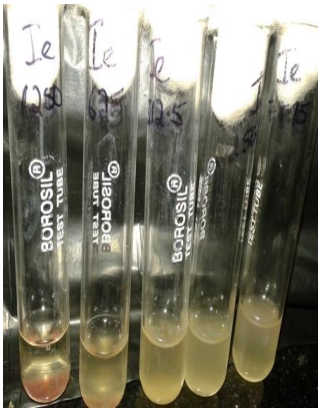


Compound: Ig

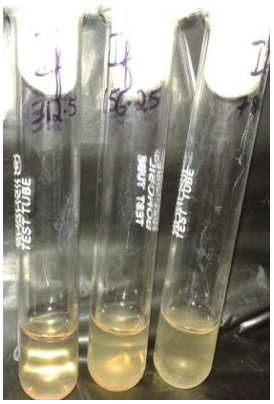
Compound: Ih

***In vitro* MIC determination against *Bacillus subtilis* NCIM 2063**

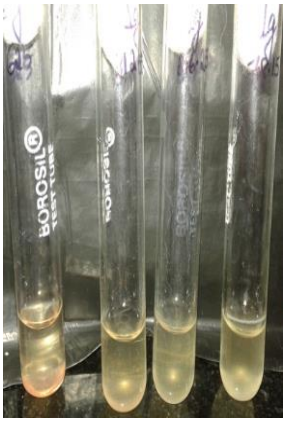
Compound : Id



Compound : Ie



Compound : If



Compound : Ig

Compound : Ih

***In vitro* MIC determination against *Escherichia coli* NCIM 2918**

Compound : Id Compound : Ie Compound : If

Compound : Ig Compound : Ih

In vitro MIC determination against _Pseudomonas aeruginosa_ NCIM 2036

Compound : Id

Compound : Ie

Compound : If

Compound : Ig

Compound : Ih

SCREENING FOR ANTIFUNGAL ACTIVITY[74]

Media - Sabouraud Dextrose Agar

Ingredients

Mycological peptone : 10 gm

Dextrose : 40 mg

Agar : 15 gm

Water to make : 100 ml

Final pH at 25^0C : 5.4±0.2

Preparation

6.5 gm of Sabouraud dextrose agar was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely.

Sterilization

15-30 ml of Sabouraud dextrose agar was transferred to petri plates and sealed. It was then autoclaved at a pressure of 15 psi (121°C) for not less than 15 minutes.

Organisms used

Candida albicans NCIM 3100 and *Aspergillus niger* NCIM 596

Drugs used : Synthesized drugs

Standard drugs : Ketoconazole(10µg)

Solvent : Dimethyl sulfoxide

ANTI- FUNGAL SCREENING BY KIRBY-BAUER METHOD

Sabouraud Dextrose Agar plates were prepared aseptically to get a thickness of 5-6 mm. The plates were allowed to solidify and inverted to

prevent condensate falling on the agar surface. The plates were dried at 37^0Cbefore inoculation.

The organisms (*Candida albicans* NCIM 3100 and *Aspergillus niger* NCIM 596) were inoculated in the plates prepared earlier, by dipping a sterile swab in the previously standardized inoculums and excess of inoculums was removed by pressing and rotating the swab firmly against the sides of the culture tube above the level of liquid and finally streaking the swab all over the surface of the medium three times, rotating the plates through an angle of 60˚ after each application. Finally the swab was pressed around the edges of agar surface. It was allowed to dry at room temperature, with the lid closed. The sterile disc containing test drugs, standard and blank were placed on the previously inoculated surface of Sabouraud dextrose agar plates. The plates were kept in the refrigerator for 1 hour to facilitate the diffusion of the drugs.

Plates were prepared in triplicate and they were incubated for 18-24 hours at 25^0C.After the incubation, the diameter of the zone of inhibition around the drugs were measured and compared with that of standard. Eight of synthesized compounds were tested for antifungal activity.

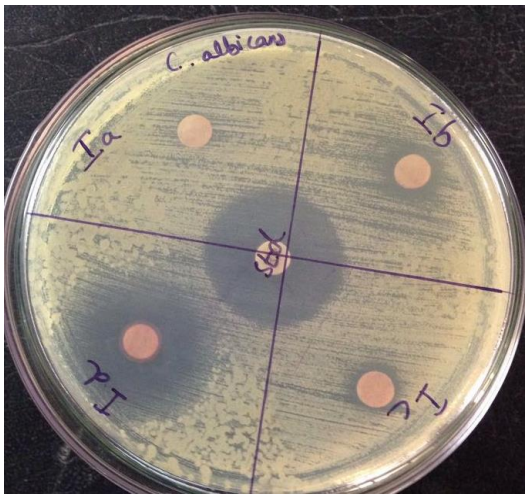
Table: 10 QUANTITATIVE SCREENING OF THE COMPOUNDS FOR ANTIFUNGAL ACTIVITY

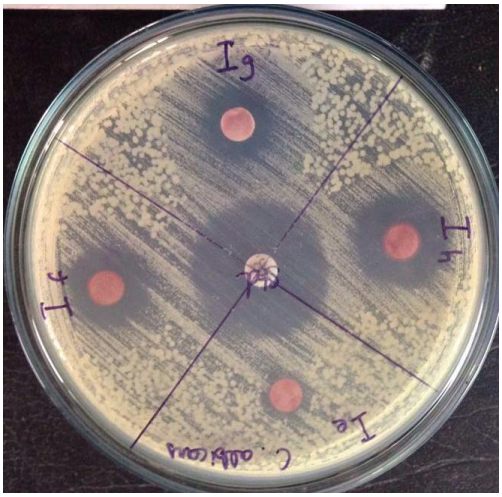
Sl No.	Compound	Diameter of zone of inhibition(mm)

	code	*Candida albicans* NCIM 3100	*Aspergillus niger* NCIM 596
		100 µg/ disc	**100 µg/disc**
1	Ia	-	26
2	Ib	12	27
3	Ic	10	30
4	Id	30	22
5	Ie	10	11
6	If	20	20
7	Ig	24	23
8	Ih	21	22
9	Blank	-	-
10	Std Clotrimazole (10µg/disc)	28	30

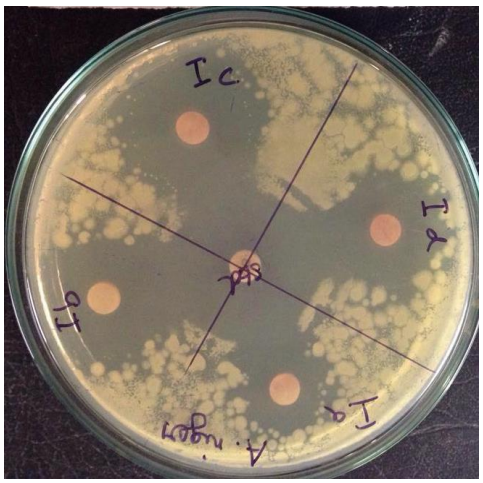
(-) indicates no zone of inhibition.

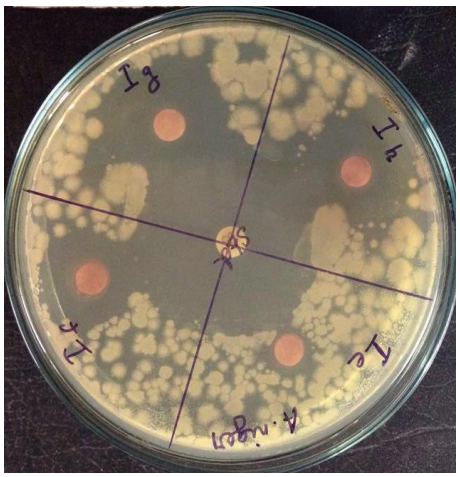
SCREENING OF SYNTHESIZED COMPOUNDS FOR ANTIFUNGAL ACTIVITY AGAINST *Candida albicans*





SCREENING OF SYNTHESIZED COMPOUNDS FOR ANTIFUNGAL ACTIVITY AGAINST *Aspergilus niger*





RESULTS AND DISCUSSION

Thymidine Phosphorylase Inhibitory activity.

All the synthesized compounds were evaluated for *in vitro* thymidine phosphorylase inhibitory activity at concentrations ranging from 10-640 µg/ml. A graded increase in the percentage of inhibition was observed with increase in concentration. The IC_{50} values were obtained by plotting percentage inhibition against concentration in µM. The values are compared with that of standard inhibitor 7- deazaxanthine.

Compounds Ia-Ih exhibited varying degrees of thymidine phosphorylase inhibitory activity with IC_{50} values in the range of 23- 154 µM.

Compound Ig (IC_{50} = 23.36±1.160) and Ic (IC_{50} =29.13±2.1) were found to be the most active members of the series, more potent than the standard.

Compound Id (IC_{50} =39.13± 3.5) and Ie (IC_{50} =41.08± 3.2) also showed excellent anti- thymidine phosphorylase activity comparable with the standard.

The compounds Ia (IC_{50} = 63.63±3.6) and If (IC_{50} =74.18±2.72) showed moderate anti- thymidine phosphorylase activity.

Compounds Ib and Ih showed IC_{50} values greater than 100 µM, therefore considered to be inactive.

Antibacterial activity

All the newly synthesized compounds were screened for antibacterial activity against both Gram positive and Gram negative organisms using Ciprofloxacin (10 µg/ml) as the standard. .

The newly synthesized compounds Ia, Ib, Ic, Id, Ie, If ,Ig, Ih were moderately sensitive against the test microorganism *Staphylococcus aureus* NCIM 2079 at 500 µg/ml concentration. The compound Ih exhibited the highest activity.

The compounds Ia, Ib, Ic, Id, Ie, If, Ig, Ih were moderately sensitive against the test microorganism *Bacillus subtilis* NCIM 2063 at 500 µg/ml concentration. The compound Ib exhibited the highest activity.

The compounds Id, Ie, If, Ig were sensitive and compounds Ia, Ib, Ic, Ih were moderately sensitive against the test microorganism *Escherichia coli* NCIM 2918 at 500 µg/ml concentration. The compounds If and Ig exhibited the highest activity.

The compound Ih were sensitive and compounds Ia, Ib, Ic, Id ,Ie, If, Ig were moderately sensitive against the test microorganism *Pseudomonas aeruginosa* NCIM 2036 at 500µg/ml concentration. The compound Ih exhibited the highest activity.

Minimum Inhibitory Concentration

The MIC values for the potent compound Id, Ie, If, Ig and Ih were determined for the test organisms *Staphylococcus aureus* NCIM 2079, Bacillus subtilis NCIM 2063, Escherichia coli NCIM 2918 and *Pseudomonas aeruginosa* NCIM 2036.

For *Staphylococcus aureus* NCIM 2079, compound Ig, If and Id showed the lowest MIC value (312.5 µg/ml) whereas for *Bacillus subtilis* NCIM 2063, compound Ih showed the lowest MIC values(156.25 µg/ml) .

For *Escherichia coli* NCIM 2918, compound Ig showed the lowest MIC value(156.25µg/ml) whereas for *Pseudomonas aeruginosa* NCIM 2036,compound Ih showed the lowest MIC value (312.5 µg/ml) .

Antifungal activity

All the newly synthesized compounds were screened for antifungal activity against *Candida albicans* NCIM 3100 and *Aspergillus niger* NCIM 596 by agar disc diffusion method using Fluconazole (10 µg/ disc) as the standard. 100 µg/ disc was used for all the test compounds.

The compounds Id, If, Ig and Ih were sensitive and compounds Ib, Ic were moderately sensitive and compounds Ia and Ie are resistant against *Candida albicans* NCIM 3100 at 100 µg/ disc concentration. The compounds Id exhibited the highest activity.

The compounds Ia, Ib, Ic, Id,,If, Ig and Ih were sensitive and compound Ie was resistant against *Aspergilus niger* NCIM 596 at 100 µg/ disc concentration. The compounds Ic exhibited the highest activity.

Table: 11 ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AT 500 µg/ disc AND ANTIFUNGAL ACTIVITY AT 100 µg/disc.

Sl No.	Compound code	Microorganism	Zone of Inhibition	Report
1	Ia	*Staphylococcus aureus*	14	Moderately sensitive
		Bacillus subtilis.	12	Moderately sensitive
		Escherichia coli	15	Moderately sensitive
		Pseudomonas aeruginosa	13	Moderately sensitive
		Candida albicans	-	Resistant
		Aspergillusniger	26	Sensitive
2.	Ib	*Staphylococcus aureus*	15	Moderately sensitive
		Bacillus subtilis.	15	Moderately sensitive
		Escherichia coli	15	Moderately sensitive

Sl No.	Compound code	Microorganism	Zone of Inhibition	Report
		Pseudomonas aeruginosa	14	Moderately sensitive
		Candida albicans	12	Moderately sensitive
		Aspergillusniger	27	Sensitive
3	Ic	*Staphylococcus aureus*	15	Moderately sensitive
		Bacillus subtilis.	13	Moderately sensitive
		Escherichia coli	14	Moderately sensitive
		Pseudomonas aeruginosa	13	Moderately sensitive
		Candida albicans	10	Resistant
		Aspergillusniger	30	Sensitive
4	Id	*Staphylococcus aureus*	15	Moderately sensitive
		Bacillus subtilis.	13	Moderately sensitive
		Escherichia coli	18	Sensitive
		Pseudomonas aeruginosa	15	Moderately sensitive
		Candida albicans	30	Sensitive
		Aspergillusniger	22	Sensitive
5	Ie	*Staphylococcus aureus*	14	Moderately sensitive
		Bacillus subtilis.	12	Moderately sensitive
		Escherichia coli	18	Sensitive
		Pseudomonas aeruginosa	15	Moderately sensitive
		Candida albicans	10	Resistant
		Aspergillusniger	11	Resistant
6.	If	*Staphylococcus aureus*	15	Moderately sensitive
		Bacillus subtilis.	13	Moderately sensitive
		Escherichia coli	19	Sensitive
		Pseudomonas aeruginosa	13	Moderately sensitive

Sl No.	Compound code	Microorganism	Zone of Inhibition	Report
		Candida albicans	20	Sensitive
		Aspergillusniger	20	Sensitive
7	Ig	*Staphylococcus aureus*	15	Moderately sensitive
		Bacillus subtilis.	14	Moderately sensitive
		Escherichia coli	19	Sensitive
		Pseudomonas aeruginosa	15	Moderately sensitive
		Candida albicans	24	Sensitive
		Aspergillusniger	23	Sensitive
8	Ih	*Staphylococcus aureus*	16	Moderately sensitive
		Bacillus subtilis.	13	Moderately sensitive
		Escherichia coli	14	Moderately sensitive
		Pseudomonas aeruginosa	18	Sensitive
		Candida albicans	21	Sensitive
		Aspergillusniger	22	Sensitive

Zone diameter : 18 and above- Sensitive

12-17- Moderately sensitive

<12- Resistant

SUMMARY AND CONCLUSION

The present work was focused on synthesis, thymidine phosphorylase inhibitory activity and antimicrobial screening of eight different Schiff bases of 7- hydroxyl- 3- formyl chromone with 2-amino 5-aryl 1,3,4- thiadiazole..

LITERATURE REVIEW

The extensive literature review reports showed that Schiff bases of 3- formyl chromone are potent thymidine phosphorylase inhibitors and the chromone moiety, thiadiazole and Schiff bases posses excellent antimicrobial activity.

SYNTHESIS

In the present work a total of eight new Schiff bases of 3- formyl chromone were synthesized.

Scheme

Step I

7- hydroxyl 3- formyl chromone were synthesized from 2,4- dihydroxy acetophenone using Vilsmeier Haack reaction .

Step II

Eight different 2- amino 5- aryl 1,3,4 thiadiazoles were prepared from thiosemicarbazide and various aryl carboxylic acid in presence of conc.sulphuric acid.

Step III

Schiff bases were synthesized from 7- hydroxyl 3- formyl chromone dissolved in methanol and various 2- amino 5- aryl 1,3,4 thiadiazoles in presence of conc. sulphuric acid.

CHARACTERIZATION

Melting points of all the newly synthesized compounds were determined. R_f values were determined on precoated silicagel –G plates by using suitable solvent systems. Structures of the compounds were confirmed by UV, IR, PMR and Mass spectral data. Yields of derivatives were in the range of 74-86%.

BIOLOGICAL STUDIES

THYMIDINE PHOSPHORYLASE INHIBITORY ACTIVITY

All the newly synthesized compounds were screened for in-vitro thymidine phosphorylase inhibitory activity. Compounds Ig and Ic were found to be the most active members of the series , more potent than the standard. Compounds Id and Ie also showed excellent anti- thymidine phosphorylase activity comparable with the standard.

ANTIBACTERIAL ACTIVITY

Eight of newly synthesized compounds were screened for their anti bacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escheria coli* and *Pseudomonas aeruginosa* by agar cup plate method at concentrations of 500 µg/ml and 250 µg/ml.

Among the compounds, Ih showed maximum activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and Ib showed highest activity against *Bacillus subtilis*. In the case of *Escherichia coli*, compounds If and Ig showed highest activity.

The compound Ih showed the lowest MIC values of 156.25 and 312.5 µg/ml for *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively. For *Staphylococcus aureus*, compounds Id, Ig and If showed the lowest MIC values (312.5 µg/ ml). Compound Ig showed the lowest MIC value of 156.25 µg/ ml for *Escherichia coli.*

ANTIFUNGAL SCREENING

All the newly synthesized compounds were screened for antifungal activity against *Candida albicans* NCIM 3100 and *Aspergillus niger* NCIM 596 by agar diffusion method using Fluconazole (10 µg/disc) as the standard. 100 µg/ disc was used for all the test compounds.

The compound Id and Ic exhibited the highest activity towards *Candida albicans* and *Aspergillus niger* respectively.

CONCLUSION

Based on the results of synthetic works, characterization data, enzyme inhibition studies and antimicrobial screening, the following conclusions were made.

- Using the schemes evolved, eight different Schiff bases of 7-hydroxy -3- formyl chromone were synthesized in good yields.
- The *in vitro* thymidine phosphorylase inhibitory activity studies indicated that compounds Ig and Ic showed excellent inhibitory activity against thymidine phosphorylase, more potent than standard. The compounds Id and Ie also showed inhibitory activity comparable with the standard. A general trend showed that more electron withdrawing substituents inserted on para position of phenyl ring will result in more potent compounds.
- The *in vitro* antimicrobial studies showed that all the compounds exhibited good antibacterial and anti fungal activity in which compound Ih showed the highest activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ib, If and Ig showed highest activity against *Bacillus subtilis* and *Escherichia coli* respectively. The compound Id and Ic exhibited the highest anti fungal activities.
- Of the 8 compounds synthesized, Ig, Ic and Id can be chosen as lead moieties for the development of effective thymidine phosphorylase inhibitors. Similarly Ih and Id can be taken up for the development of ideal anti bacterial and anti fungal agents respectively.